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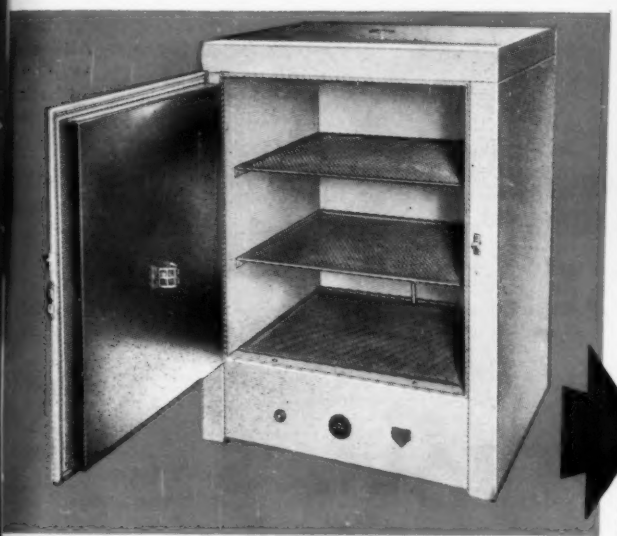

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Picture by courtesy of Douglas Lawson, F.I.B.P, F.R.P.S, F.R.M.S, F.Z.S. Author of 'The Technique of Photomicrography'

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EDUCATION FOR AN INDUSTRIAL SOCIETY

So many conferences of a self-duplicating nature are arranged by educationists in industry that the question often arises as to what the organizers do when they are not taking in each other's washing. As recent reports show, the British Association for Commercial and Industrial Education has not been entirely free from the criticism that some of the many conferences it arranges are but repetitions of previous ones and attended by the same round of converted disciples[1]. It is gratifying, therefore, to direct attention to the Association's annual conference which was held at Cambridge during September 22–24, 1960[2]. This was the kind of conference which could not fail to stimulate those attending; there was a co-ordinating theme which was readily apparent and the active participants were there because they—nearly all—had something to say, and said it with conviction. In addition, the organizers had prepared advance copies of each paper containing, as introduction, a summary of a recent Government report which the speaker had been invited to consider. As a result conference members were presented with the opportunity to examine the whole broad front of educational advance and the way it could affect young people in our industrial society.

The Albemarle, Crowther, McMeeking, Carr, Anderson, and Willis Jackson Reports were all considered, as were the work of the Industrial Training Council and the Government's new proposals for management studies. With one exception, each speaker was not only able to present a review from intimate knowledge of a report but also was able to illuminate passages which had hitherto been dark.

The Albemarle Report, for example, was considered by M. J. W. Clapham, a director of Imperial Chemical Industries, Ltd., who showed how a sceptic came to support the ten-year development programme for young people put forward by an enthusiastic committee. The approach itself is one which should commend itself to all who are appointed to important national committees and may well explain why the Minister of Education was able to announce his acceptance of the committee's proposals on the same day that the Report was published.

It was unfortunate that a Report which has been far less favourably received—and especially by industry—should not have been presented in a similar manner. Instead, Miss M. G. Green, a member of the Central Advisory Council for Education (England), which produced the Crowther Report, chose simply to summarize its main recommendations for those who cannot or will not read. So much controversy has arisen over the priorities and timings of the Committee's main recommendations that those who support them should not throw away opportunities to gain public sympathy; many industrialists believe that the Crowther Committee has mixed up its priorities and has produced a Report lacking urgency and conviction.

The Carr Report on apprenticeship training was considered particularly in relation to its foster-child, the Industrial Training Council. While Lord McCorquodale, chairman of the Industrial Training Council, strove loyally to extol the work of his gently moving organization, the revelations of the Minister of Labour, the Right Hon. John Hare, showed that industry has failed lamentably to accept the Government and Industrial Training Council exhortations to provide greater facilities whereby school-leavers can systematically learn the skills of a craft. From the Minister's address it became apparent that the short-sightedness and remissness of employers could, inside a generation, starve the country of the skilled and adaptable workers who are essential to an economy based on science and technology. The Government has already intervened by providing places for three hundred apprenticeships, and the portents are that, with the failure of industry, massive Government intervention is inevitable.

Reviewing the Report which bore his name, Sir Colin Anderson did not hesitate to speak "between the lines", and made it clear why the majority of his Committee believes that the way to the university should no longer be controlled by the means or wishes of the parents. Further education should be something which the student can secure for himself by the use of his own brains, initiative and energy, even if his parents are uninterested.

The danger confronting the universities, and especially the older foundations, in becoming public trustees for the higher education of the nation—which implies much of its financial as well as other resources—was also examined by Sir Colin, who showed that the universities need lose none of their spiritual autonomy in a free, industrialized society.

The sponsors of the conference may have combined joint consideration of the McMeeking Report with the new scheme for management studies for their own good reasons. Despite the garnishing, the dismal failure of efforts to build up the National Certificates in Commerce since the publication of the McMeeking Report some eighteen months ago has led to considerable speculation about the Government's rejection of a Working Party's recommendations to develop an Intermediate Certificate in Management Studies and its virtual substitution by the new Higher National Certificate in Business Studies. Many members of the conference felt that the Ministry of Education

had placed administrative convenience before the genuine needs of management students and was determined to push the latter into courses in business studies. This, no doubt, will shore up the precarious courses in business studies by providing enough students and teachers to make classes worth running. Mr. J. C. W. Stead, a director of Batchelor Peas, who was a member of the Working Party appointed to examine the Scheme of Management Studies, was right to insist that a fundamental mistake had been made by the Ministry in trying "to combine a course which purports to help people to an understanding of management principles with a course which sets out to attract young people, in some cases straight from school, at an age of 18, for the purpose of studying certain commercial subjects". Before proceeding with this irreconcilable union, the Ministry should seek the advice of the Committee set up under the chairmanship of Sir Keith Joseph to consider the establishment of courses in management studies at one or more universities. The Ministry is revealing the same attitude to management studies as it did to science education some forty to fifty years ago. It should encourage part-time students who come up the hard way to extend their knowledge with studies of management principles and practice ; such students bring qualities to industry which are not always present in management trainees recruited from graduates.

Sir Willis Jackson dealt in an exemplary manner with his own Report on the Supply and Training of Teachers for Technical Colleges. He described separately the proposals contained in the Report and action taken afterwards. The pay of full-time technical teachers, for example, was raised by the Burnham Committee in 1959 and has been followed by appreciable increases in the scales of part-time teachers. Consultancy work is beginning to develop in the advanced colleges, and more teachers are following courses of study leading to higher qualifications. The supply of part-time lecturers of standing to such day students is slowly increasing but the disparity in salaries is proving a considerable barrier to the transfer of senior scientific Civil Servants. The short courses for training serving teachers to teach better have been extended, and a new teachers' training college is being developed at Wolverhampton. Enough money has been raised to establish the Staff College for technical teachers and the location of the College and appointment of the director of studies should shortly be made. Sir Willis also described the arrangements for increasing the total number of teachers for technical institutions and deserves considerable praise for the part he is playing in this.

The conference was one which the British Association for Commercial and Industrial Education can feel proud to have organized. Those attending should now see that the weighty matters discussed are not allowed to be forgotten in their local activities.

[1] Education for Survival. Report of the Ninth Annual Conference of the British Association for Commercial and Industrial Education, East Midlands Group. The Implications of the Crowther Report. Report of the B.A.C.I.E. Spring Conference.

[2] Policy in Perspective. Annual Conference of the B.A.C.I.E., 1960.

LIFE IN THE SOIL

Living Earth

By Peter Farb. Pp. ix+178+8 plates. (London: Constable and Co., Ltd., 1960.) 21*s*. net.

ON learning, from the inside flap of the dust-jacket, that the author of this book "was formerly a Feature Editor of *Argosy* magazine", and further, from the author's own preface, that his "approach has been that of the science reporter", the intending reader might feel (as I did) some slight apprehension lest he should be in for a piece of journalistic pot-boiling. But he would be quite wrong. This is a soberly, sanely and attractively written work, presenting a comprehensive and often entertaining picture of what is at present known of the teeming inhabitants of the soil, and of their varied conditions of life. Even for many readers with a broad knowledge of natural history it will certainly provide an introduction to an entirely new world ; and for once—*mirabile dictu*—a reviewer may be permitted to agree with a publisher's blurb, when it uses such enthusiastic expressions of approval as the "fascination" and "excitement" of the book.

Mr. Farb is an American, living in the State of New York (he was formerly secretary of the New York Entomological Society), and his book has naturally a New World emphasis. He discusses the life of the soil under the three main headings of "Forests", "Grasslands", and "Deserts", with an introductory section on "The Living Soil", and a concluding section on "Mutual Survival". A four-and-a-half page descriptive bibliography and a (not entirely adequate) index complete the work.

One is tempted to quote liberally from a book that is so full of good things. A feature that especially commends itself is the author's emphasis on the interdependence of all forms of life, their intimate mutual relationships, and their complex interactions with their environment, forming with it one indivisible whole. Of certain 'wayfarers from the soil' he writes : "We can but divine their rôles in the intricate web of grassland life ; for no creature of the soil stands alone—all are interwoven, their pasts and their futures, in the economy of a piece of earth" (p. 130). "The dividing line between animals and plants is often a slim and artificial one . . . The line between living and non-living forms is not so sharp as [was] once supposed" (p. 15).

The author has the valuable gift, in the midst of his factual reporting, of lifting his eyes to wider horizons and pointing out some of the implications of the facts he records. In the course of his remarks on the soil-dwelling social insects, to which he devotes much space, he says of the termites : "Termites are a very ancient form of insect life . . . going back well over 250 million years . . . In their great age, termites question one of the popular beliefs about evolution—that all living things on the planet are progressing toward more perfect organisms. Termites reached their full structural and social development, including their very complicated system of castes, in the Tertiary period, many million years ago. Since then, they have neither advanced nor fallen by the wayside, but now populate the earth in their age-old pattern" (p. 83). "Many experimenters have looked upon the termite nest as actually a superorganism in which each of the castes functions as a part of a single, larger organism . . . The superorganism theory . . . has many biological obstacles

to meet . . . yet in the hot termite mounds may lie an answer to one of the most perplexing questions about life itself" (p. 91). How faithfully certain aspects of human activity are sometimes mirrored in the lives of creatures allegedly 'lower' in the scale! One of Mr. Farb's most dramatic and vivid passages is that in which he describes the seasonal sequence of events in a wasps' nest (pp. 91–94). Towards the end of the summer, after the departure of the new queens and kings, "a madness begins to eat away at the very foundations of the city . . . workers take to devouring the larvæ ; but still unsated, they abuse, then torture, the young. An orgy has begun that will end only with the death of the city . . . They rush through the combs looting and destroying. The queen is assassinated, and every worker becomes a queen. It is democracy in its most horrible form."

The interesting and important part played by root secretions, or 'diffusates' (this word, incidentally, is missing from the index), is touched upon in many places. While their primary function is no doubt to provide some kind of benefit to the owner of the root—for example, the encouragement of the growth of symbiotic or otherwise beneficial fungi and bacteria in its immediate vicinity, or the prevention of the growth of certain other plant competitors within a certain radius—it is evident that many other organisms have 'cashed in' on this secretory activity of root-systems for their own ends. It is found, for example, that root diffusates exercise a positive attraction to wireworms, the larvæ of click-beetles (Elateridae), which use the roots as food ; that they act as a stimulant to the awakening of encysted nematodes, which then enter the root and commence their work of destruction ; and that they also provide the stimulus for the germination of the seeds of certain pernicious root-parasites, such as *Striga* (Scrophulariaceae). It is clear that these chemical substances are one of the most vital factors in the complex interactions of organisms in the soil.

A few points call for criticism. The photographs are, on the whole, very good, but the text-figures are often poor. No list, either of plates or of text-figures, is supplied. Misprints are commendably few—curiously enough consisting almost entirely either of singulars for plural, or the reverse : the fractions on p. 67 ("1/100ths", etc.) are very odd. Some startlingly untrue statements are clearly slips : "the most obvious part of the fungus . . . is its vegetative organ, the toadstool" (p. 66) ; "this animal [the Mycetozoan swarm-spore] divides a few times by simple fusion" (p. 71). Some may have been due to misunderstanding of informants : "most orchids have little or no chlorophyll" (p. 63). Certain remarks betray carelessness of phraseology or looseness of thought : "Alfred R. Wallace . . . co-discoverer with Darwin of the theory of evolution" (p. 75) (How does one 'discover' a 'theory' ?) ; "Linnaeus, the Swedish botanist who invented the modern method of classifying living things into various categories" (p. 83). But there are one or two remarks over which I am still scratching my head. "This mold [the Mycetozoa] may well be 'the slime of the earth' of the Book of Genesis" (p. 70). Has Mr. Farb had access to a new and shattering Hebrew manuscript ? Or has he merely got a bit mixed between Genesis ii, 7 and xi, 3 ? "On a beastly summer day in South Africa . . ." (p. 84). Never having been in South Africa, I have no idea whether its summer can be really as unpleasant as the author seems to imply ; but his scathing epithet seems to rise from the page with the impact of a blow to the solar plexus, leaving one slightly winded. Finally : "Dr. Livingstone, before being hounded by the intrepid Stanley, was an enthusiastic naturalist" (p. 84). Poor, meek, weak-minded Livingstone ! Poor, splendid, overbearing Stanley ! Do they really deserve these aspersions ?

But it is not necessary to end on a frivolous note. Mr. Farb has written a most interesting, thought-provoking and worthwhile book.

H. K. Airy Shaw

THE STRUCTURE OF BACTERIA

The Bacteria

A Treatise on Structure and Function. Edited by I. C. Gunsalus and Roger Y. Stanier. Vol. 1 : Structure. Pp. xiv+513. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 13 dollars.

"HERETOFORE, there has been no comprehensive source of information about modern knowledge of the general biological properties of bacteria." This statement of the editors is the clue to this volume and, presumably, to the four others projected which will cover the fields of metabolism, biosynthesis, growth and heredity, and will be welcomed by the growing legion of bacteriologists whose interests lie primarily in the bacteria themselves and not in the immediate medical or industrial applications of bacterial properties.

There are ten chapters, each contributed by an internationally distinguished worker, which bear the following headings : "The Bacterial Protoplasm—Composition and Organization" ; "The Internal Structure of the Cell" ; "Surface Layers of the Bacterial Cell" ; "Movement" ; "Morphology of Bacterial Spores, Their Development and Germination" ; "Bacterial Protoplasts" ; "*L*-Forms of Bacteria" ; "Bacterial Viruses—Structure and Function" ; "Antigenic Analysis of Cell Structure" ; and "Localization of Enzymes in Bacteria". Chapter 1 is a general introductory survey.

As might be expected in a treatise by many authors total co-ordination was difficult, but it is perhaps a feature of strength rather than weakness that some overlapping occurs. Occasionally, seeming contradictions have crept in, as : "Even the processes involved in all forms of motion of living organisms seem to share a common basic metabolism ; stimulation of contractile protein with ATPase activity by the common substrate adenosine triphosphate" (p. 4), while the same author on p. 26 states, "No report of ATPase activity in flagellar protein has been made". The enigma is partially solved in the section on movement (p. 173), "Not very much is known about the energy supply of active flagella. . . . De Robertis and Franchi claim that bacterial flagella contract in the presence of ATP. De Robertis and Peluffo have found that ATP enhances the motility of *Proteus vulgaris*. On the other hand, Barlow and Blum found no ATPase activity in flagella preparations". Such conflict is the seed of science, and as the text is generously impregnated with references (more than 1,500) the reader can easily discover the sources of the claims made and judge them for himself.

The authors have refrained from dogmatism, and long-standing disputes such as the nature of bacterial

nuclear material, the method of its division and whether flagellar movement is the cause or the result of the movement of the organism, are treated impartially. The chapter on bacterial viruses appears short when viewed against the activity and interest in this field—less than twenty pages excluding illustrations. Many of the illustrations included in the volume are outstanding examples of the advances made in technique, especially with the electron microscope.

The various topics are clearly sub-headed and used in conjunction with the detailed contents, and author and subject indexes make this a very accessible reference work and augurs well that the completed treatise will be the most significant addition to bacteriological literature for some years. It will also be expensive.

L. B. Quesnel

GENES AND PROTEINS

The Molecular Basis of Evolution

By Christian B. Anfinsen. Pp. xiii+228. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 56*s*. net.

At first glance, some features of this book are likely to seem a bit disconcerting. At the present time there are three fields of inquiry which have considerable claims to be considered, both particularly active and peculiarly important for general biology: one is the evolutionary process; the other two, which are closely connected, are the sub-structure of genes, and the configuration of the protein molecule. Dr. Anfinsen seems to be claiming to have gathered all three together into the confines of a comparatively small book. Its dust-cover states that he "regards the evolutionary process as the integrated form of genetics and protein chemistry". Unless the word 'genetics', which is a notoriously elastic one, is here taken to include such matters as natural selection, mutation, species divergence and so on, this way of regarding evolution would certainly seem to be a bad case of Hamlet without the Prince of Denmark.

However, it would be a pity to take these first impressions too seriously. Apart from reproducing a few well-known figures from a book by G. G. Simpson in the first chapter, and giving a short outline of elementary genetics in the second, the author in fact pays little attention to the subject of evolution. What he has actually produced is a summary of recent work on the relation between the sub-structure of the genes and the proteins which they specify. Most of this account follows the lines which are already becoming recognized as orthodox. For example, in the description of studies of the mechanism of chromosome duplication with the aid of radioactive labels, Anfinsen describes the work of Taylor and his associates, in which the chromosomes did what they had been expected to do, but does not mention that of Plaut and Mazia, who came to different conclusions, although he does add a footnote concerning the results of La Cour and Pelc, which also differed from those of Taylor.

The writing in this part of the book is rather slapdash and not very accurate. For example, in discussing Taylor's work on chromosome duplication on page 59 the author writes: "In an occasional 'second generation' pair of chromosomes, individual sister chromatids were labelled for only part of their length . . . and in such cases the corresponding portion of the other chromatid showed radioactivity. This behaviour is to be expected for crossing-over and resembles the cytological observations that have been made for the chromosomes of other species (e.g. the giant salivary gland chromosomes of *Drosophila*)". Actually the situation is that when crossing-over occurred in Taylor's chromosomes, and a chromatid was found to be labelled for only part of its length, the corresponding part of the sister chromatid did *not* show radioactivity. Moreover, no phenomena comparable to crossing-over occur in the giant salivary gland chromosomes of *Drosophila*. Again, to give another example, on p. 88 Anfinsen writes: "As we discussed in connection with the 'lozenge' genes of *Drosophila* (Chapter 2), three closely linked genetic loci all appear to be part of the same functional unit since crosses between double heterozygotes bearing both mutant loci on the *same* strand of the chromosome duplex (i.e. in the *cis* arrangement) produced wild-type recombinants, whereas crosses between double heterozygotes in the *trans* arrangement did not". This sentence suggests a state of complete confusion. In the first place the phrase 'chromosome duplex' is normally used to refer to the two helically twisted strands which together make up the deoxyribonucleic acid molecule; and the point at issue is not whether the mutant loci are on the same or different strands in such a duplex, but whether they are on the same or different chromosomes. Then the well-known '*cis-trans* criterion', to which Anfinsen is referring, does not turn on the nature of the recombinants which may be produced by crossing the double heterozygotes, but on the simple fact whether the double heterozygote itself shows the mutant character or not. Anfinsen actually describes this quite correctly in Chapter 2. Presumably the confusion that has arisen in the passage quoted was due simply to some lapse of attention.

It is when we pass from the more genetical parts of the book to the more chemical sections that its true value begins to emerge. Anfinsen is himself a protein chemist, and his handling of the chemical aspects of his subject is much more confident than that of the genetical material and, at least to a genetical reader, carries much more conviction. He provides an admirably clear account of recent work on the analysis of the sequence of amino-acids in various proteins, and of attempts to unravel their secondary and tertiary structures. This is illustrated with a large number of very clear diagrams. It will undoubtedly be extremely useful and suggestive to many biologists who are interested in this topic without being expert in it. Thus, in spite of certain inadequacies, therefore, his book has very real value.

C. H. Waddington

GENETIC ANTHROPOLOGY

The Mothers

By Robert Briffault. Abridged, with an Introduction by Gordon Rattray Taylor. Pp. 451. (London: George Allen and Unwin, Ltd., 1959.) 36*s*. net.

The re-publication of "The Mothers", reduced from more than two thousand pages to less than five hundred, is intended to make it available to "the maximum possible public". It is doubtful, however, whether any great interest in it will be aroused among social scientists, except as an example of a

phase in the development of anthropology. There is a fundamental difference between an attempt such as Briffault's to reconstruct the hypothetical course of the development of the institution of the family over the ages, and modern anthropology, *pace* Prof. Gordon Childe and Dr. Freud. Indeed, genetic anthropology is, in Prof. Evans-Pritchard's phrase, now generally regarded as an "attempt to explain what we know something about by what we know next to nothing about"; the reaction against it occurred at the beginning of the present century, and developments since then have been along quite different lines.

On the other hand, the controversy between Briffault and Westermarck, which was waged with such bitterness a generation ago, still has a direct interest for us to-day. The immediate issue at stake is no longer a live one; it centred round the problem whether the origin of the family is to be found in a primitive matriarchy (as Briffault thought), or in the activity of masculine jealousy and possessiveness and therefore, as Westermarck held, a patriarchy. The significance of the conflict for the contemporary social scientist is in the manner in which the conclusions of each of the protagonists was built into his work by his primary assumptions; this also determined the selection of the evidence which was discussed as well as the interpretation placed on it.

"The Mothers" may thus be regarded as a brilliant piece of special pleading, and may well live in the literature of the social sciences as the classic example of illustrative or interpretative, rather than truly analytic, work. It may also serve a very valuable purpose indeed as proof of the fact that a text in the social sciences can be written with brilliance, and captivate the interest of the reader. Information collected from the four corners of the earth must be used for scientific purposes with the greatest caution, but it does give the thesis it illustrates liveliness and colour for all that. By no means the least of the merits of this abbreviation of the text is the extent to which it preserves the literary qualities of the original work.

T. S. Simey

PROGRESS IN THE STUDY OF VISION

Physiology of the Retina and the Visual Pathway

By G. S. Brindley. (Monographs of the Physiological Society, No. 6.) Pp. xi+298. (London: Edward Arnold (Publishers), Ltd., 1960.) 35*s*. net.

THIS thoughtful and provocative book provides a critical synthesis of the present state of a wide range of visual physiology and biochemistry. Its primary interest is with human vision. Though written by one of the younger workers in the field, it is in large part concerned with matters with which he has already established direct experimental contact; for since 1952, when his first papers appeared, Dr. Brindley has made important contributions in many areas of visual investigation.

A special characteristic of the present volume is its attempt to formulate explicitly the logical structure that underlies areas of sensory investigation in which most previous workers, with notable exceptions, have been content to muddle through to whatever solutions seemed reasonable to them at the time. This is a noteworthy and laudable effort, bound to have an effect, however time deals with the specific arguments. Indeed Brindley makes no attempt to create an illusion of finality. He is anxious to expose whatever argument he makes in all its strengths and weaknesses, frequently stressing the latter. The field of vision is now developing rapidly, and the present volume maintains a continuous impression of work in progress. Again and again Brindley leads the reader into some present puzzle, and ends with the reassurance that, judging by the present rate of progress, more clarity may be expected shortly.

Perhaps I have already conveyed the impression that this is a highly individual view of the subject. It is perhaps the most wide-ranging attempt to produce order in this field, by a consistent exercise of logical rigour tempered with good sense, since Helmholtz. Indeed my few complaints in reading the book came from the occasional sense of being shut out. Brindley now and then, though rarely, announces some awareness that the reader is presumed to share, since no attempt is made to explain it, but which the person whose work is under discussion apparently missed. A good example occurs on p. 199, where Newton is said to have "published a diagram for predicting the results of colour mixture which, if it were exactly valid (which it clearly is not), would imply trichromacy . . . Newton . . . did not point out its implications". What sticks here is the "would imply trichromacy". Newton's diagram had seven components, to simulate the seven tones of the musical scale. Brindley sees an implication of three primaries, probably with good reason, but does not stop to explain.

Another such passage strikes closer to home. On p. 184 Brindley says: "A mechanism which would achieve something like a suitable relation was suggested by Wald (1954): a rod might consist of many compartments, each inactivated by the photolysis of any one of the molecules of rhodopsin in it. In its original form, this suggestion is contradicted by the very data that Wald used to support it". I should certainly like now to change some parts of my original presentation of this theory; yet I do not know what contradiction Brindley has in mind, and he does not say.

The book opens with what is, on the whole, an expert and searching review of retinal chemistry. This chapter, and Chapter 3 on the central pathways of vision, represent areas with which Brindley has had least direct contact; yet here as elsewhere in the book he presents a valuable critical synthesis of the data. Chapter 2 discusses the electrical activity of the retina. Its main points are the acceptance of Brown and Wiesel's identification of Bruch's membrane as the high-resistance structure across which the electro-retinographic potential is built up, replacing Brindley's earlier idea that this is the external limiting membrane; and Brindley's present conviction that the bipolar cells of the retina are the dominant source of the electroretinogram.

Chapter 4 introduces the discussion of the sensory experiments that occupy the remainder of the book, with an analysis of the logical structure underlying such experiments. All such observations involving reports by experimental subjects contain psychological as well as physical components, which must be connected, implicitly if not explicitly, by what Brindley calls "psychophysical linking hypotheses". He distinguishes class *A* observations, which involve only statements of equality or non-equality of sensations, from class *B* observations, in which a sensory judgment must be made, demanding some

special evaluation, interpretation, or abstraction from the total experience. Brindley discusses the limits of usefulness of class *B* observations, and ways of improving their status. He then applies this logical apparatus to the analysis of a variety of entoptic phenomena, after-images, contrast phenomena and electric phosphenes (Chapter 5); functions involving visual thresholds and adaptation (Chapter 6); and colour vision (Chapter 7).

Brindley characterizes this volume in his preface as "a tightly written book, very full of information and for this reason not easy to read from end to end consecutively". This is fair enough, though I should add that it repays handsomely whatever trouble one takes with the reading. A few typographical errors at times make special trouble: in Fig. 2·4 (p. 50), the legend says 155μ when it means 255μ; and in Fig. 2·14, the legend should read "In *a*, taken at 140μ from the outer ends of the receptors, a large response is recorded from the *inner* pipette, but almost none from the *outer*".

George Wald

THE SEPARATION OF ISOTOPES

Electromagnetic Isotope Separators and Applications of Electromagnetically Enriched Isotopes

By J. Koch, R. H. V. M. Dawton, M. L. Smith and W. Walcher. Pp. xi+314. (Amsterdam: North-Holland Publishing Company, 1958.) 26.50 guilders.

THE separation of isotopes on a large scale was achieved by several methods during the Second World War as a necessary stage in the manufacture of the atom bomb. Of the various methods employed the electromagnetic separator (calutron), based directly on the mass spectrograph principle, was able to be brought to the production stage earliest. The uranium-235 for both the Alamogordo test explosion and the Hiroshima bomb came from the electromagnetic separators. After the War the far more economical gaseous diffusion process was brought into large-scale production so that the calutron process became redundant for the production of uranium-235.

Nevertheless, the many uses for comparatively small amounts of separated stable isotopes of a wide variety of elements which can be obtained most conveniently from electromagnetic separators has kept an interest in the subject alive, so that many laboratories all over the world have acquired such separators in recent years. The availability of samples of almost pure individual isotopes greatly facilitates the work of the nuclear physicist in working out the details of nuclear interactions, in assigning activities to particular isotopes, and in the investigation of the validity of different nuclear models. It is of importance in spectroscopy where it has enabled the elucidation of the finer details of spectra previously masked by the presence of several isotopes, and where it has made possible the achievement of greatly improved wave-length standards. Such separated isotopes have also been used in solid state physics and in biological studies.

The present work consists mainly of a survey of the construction of such practical separators and of the physical principles of their operation. It also includes a very brief discussion of some of the applications of the enriched isotopes produced by the method. It confines its attention to the calutron type of apparatus so that it is not comprehensive in the discussion of all types, even of electromagnetic separators. There is no discussion, for example, of the omegatron. It is divided into three parts, each of about equal length. The first part, written by J. Koch, is devoted mainly, but not exclusively, to separators developed largely independently of the American experience gained during the Second World War. These were mainly developed after the War, but since the American experience was not declassified until the early 'fifties, independent development was necessary.

The second part, by R. H. V. M. Dawton and M. L. Smith, is mainly devoted to the description of the Harwell separators which were constructed in full knowledge of the American war-time experience. The method of treatment necessarily entails a considerable overlap between these two articles. They are both concerned mainly with technical constructional details rather than physical principles and will therefore have a rather restricted appeal.

The physical principles of the ion-optical systems employed, the high-current sources and the fascinating problems raised by the space-charge neutralization of ion beams are discussed by W. Walcher in the third section, which will be of more general interest. The book should prove of great use for those interested in designing isotope separators while the third section will be of interest to wider circles of physicists.

E. H. S. Burhop

EDUCATING YOUNG NATIONS

Educating Young Nations

By W. E. F. Ward. Pp. 194. (London: George Allen and Unwin, Ltd., 1959.) 15*s*. net.

A MAN who took Beethoven's Fifth Symphony to the Gold Coast thirty-five years ago, and made that music understood, and who to-day writes of the necessity to foster, through education, common nationhoods, which yet respect cultural diversities, has within him a Greek sense of balance and a Christian humanity. These qualities make Mr. Ward's book memorable, kindly and historical. It will be of political and professional value to administrators and educationists working in the newly independent countries of Africa and Asia which have grown up overnight.

Mr. Ward wrote this book in 1959. It is now past history which will interest the newcomers to the field of public service. To those who shared the experience it will provide nostalgic delight. The validity of the thesis that education, particularly the education of a reserve of good teachers, "everyone of whom will be needed when the day of expansion comes", has already been proved. The plea for European technicians to stand by, while the new nations find their feet, is already the theme of the speeches of the more balanced politicians. The proposal that money for education should be looked upon by governments as aid to development rather than to welfare projects, commanding assistance "as if it were a scheme for irrigating the Sahara" is likely to gain the sympathy of the new Ministries of Finance.

Mr. Ward is probably the last author to write contemporarily of "the Colonies" and of "expansion" as a synonym for "independence". His catholic experience of teaching and administrating in places as dispersed as the old Gold Coast and Mauritius, his representation of the interests of those countries in the

United Nations, his work as a senior adviser on education to the Secretary of State for the Colonies, give him an authority which will be a reassurance to those still serving that nothing is too difficult for courage and that good human relationships are still more important than politics or race.

The major educational issues with which the book deals—poverty, syllabuses, language, the education of women, selection for secondary work, multi-racialism and teacher-training—will continue to find their various experimental solutions; but the need of humanity for humanity is timeless. There is a charming parable of interdependence in the story of the student offering his notes for a school practice lesson on "The Elephant, a Pachyderm". The apparatus listed was "one elephant, one pin", the latter to prove the toughness of the elephant hide. "Have you your pin?". "Yes Sir, here it is". "And your elephant?" "Sir, as for that, I thought that you could help me".

Mr. Ward points out, soberly enough, that the enormous growth in demand for education, off-set by lessened recruitment from the United Kingdom, is likely to result in a temporary loss of standards in schools. He records the political and social implications of so-called education by teachers who are "mainly chalk and talk" and stresses that, so far as possible, quality should be maintained at the top. One is left guessing at the contents of the unwritten chapter on the influence of the new leadership on education and the length of time during which, with genuine trust and expectation, those who are achieving independence will continue to look to an older order for the production of the elephant.

MIRIAM JANISCH

A HISTORY OF METALS

A History of Metals

By Leslie Aitchison. Vol. 1: Pp. xxi+1–304 (figures 1–127). Vol. 2: Pp. xvii+305–647 (figures 128–262). (London: Macdonald and Evans, Ltd., 1960.) 168*s*. the two volumes.

"A HISTORY OF METALS" differs from many others on a similar theme, first in that it has been written by one who is a metallurgist of wide experience and high reputation, qualifications not found in all writers on the subject. In the second place, the author has painted his picture against a background of the history of civilization itself, which both provided the conditions in which metallurgy could develop and which, on the other hand, were themselves fundamentally modified by those very developments. The appeal of these volumes should, therefore, be not only to the metallurgist but also to the archæologist and social historian.

In the author's treatment there emerges little which is essentially novel; in his own words the book is "a fractional distillation" of the work of others. His duty has been to collect facts already known, to sift the wheat from the chaff, and then to present a reasoned, critical account of his harvest. It follows, therefore, that he has inevitably been dependent on others for his original data. Cross-linkages, however, are not infrequently possible which strengthen the main structure and give it a stability in so far as the chief features are concerned. Details, at times even important ones, particularly those relating to the earlier stages of development, are still uncertain, and not everyone will agree with the author in all particulars. This is perhaps especially true, as he makes abundantly clear, in connexion with any attempt to provide more than an approximation to actual dates.

These volumes cover a period of some six or seven thousand years of which Volume 1 carries the story down to about 1000 A.D. and deals with the seven metals gold, copper, silver, lead, tin, iron and mercury together with their alloys bronze, brass, pewter, steel and amalgams. "Broadly, the several metals and alloys were used for much the same purposes at the end of this long period as at its beginning the evidence of development appearing more in the variety of articles made . . . than in novel applications. As the centuries passed, technological improvements in metallurgical processes were effected and greater weights of material were handled, but during the whole of the . . . years dealt with . . . metals were won and worked by manual power. . . . Appropriately, therefore, Volume 2 opens at the time when power that has not been generated by human muscles was first applied to metallurgical operations", in any event on a substantial scale. The processes by which the metals were extracted from their ores, however, remained essentially unchanged for another two or three centuries at least. As Dr. Aitchison points out, "when the thirteenth century opened, iron—in most parts of Europe and certainly in England—was still being extracted by almost exactly the methods that had been used three millenia earlier" and much the same is true of the other metals.

In the author's view a metallurgical medievalism then occurred which lasted until the beginning of the eighteenth century, though these five hundred years were by no means static. From a craft an industry was relatively rapidly evolved, a medieval industrial revolution. It may well be that Dr. Aitchison's main contribution is to be found in the three chapters which, starting with the Crusades and followed by the discovery of the American Continent, deal with this period.

It is not surprising that an author, most of whose life has been spent in industry, should turn his readers' attention to commerce as well as to technology. His chapter on "Traders from Troy" draws a picture of the commerce of the third millennium B.C. of particular importance from his point of view in its profound influence on the resulting dispersion of knowledge of the technologies in general.

The predominating importance of the metals in moulding and stimulating scientific thought in all ages is reflected in three chapters concerned with this aspect of his history, first in antiquity, then during the birth of modern science and finally in the present era of nuclear transformations. Although the later parts will be well known to scientific readers, Dr. Aitchison justifiably stresses the part played by technological processes and products in the evolution of our scientific and philosophical ideas.

No review of these volumes would be adequate which did not emphasize the author's easy presentation of his material and his skilful avoidance of technicalities. The excellent illustrations have been well chosen, and many, in any event, will be new to most readers: there are a list, comprehensive but not exhausting, of sources for further reference and a good index. The price is high, but those who are able to acquire this book will have a treatise well worth possessing.

F. C. THOMPSON

Sedum of the Trans-Mexican Volcanic Belt

An Exposition of Taxonomic Methods. By Robert T. Clausen. Pp. x+380. (Ithaca, N.Y.: Comstock Publishing Associates, Cornell University Press; London: Constable and Co., Ltd., 1959.) 65*s*. net.

THIS very thorough investigation of 28 species of *Sedum* growing in the Trans-Mexican Volcanic Belt should be noted by all those interested in modern taxonomic methods entailing population studies in the field and experimental garden as well as the examination of herbarium specimens and literature. The author's conclusions, in his own words, are based primarily on gross morphology and ecology: only limited data about chromosomes, anatomy, embryology, etc., were available. "Such data, when used to overrule conclusions based primarily on details of gross morphology and ecology, should be based on samples sufficiently large to be of statistical significance." His own approach to the problem of sampling and the study of environmental modifications should be read by everyone working on revisions of critical north temperate plants, and his comparison (Chapter 8) of his own work on *Sedum* with that of his predecessors, Berger and Fröderström, is revealing. *Sedum* was a good genus to choose for attack by modern taxonomic methods: material is easy to propagate for cultural experiments, and "dried specimens of *Sedum* in herbaria are not reliable for descriptive data, regardless of how valuable they may be for indicating the distribution of species".

N. Y. Sandwith

Heredity of the Blood Groups

By Prof. Alexander S. Wiener and Irving B. Wexler. Pp. x+150. (New York and London: Grune and Stratton, 1958.) n.p.

DURING the past fifteen years, as the authors point out, knowledge of the blood groups has progressed with great rapidity, and blood grouping has become a fundamental pathological science, knowledge of which is important in many fields. The heredity of the blood groups should be clearly understood, and the authors in this book set out their conception of this; but the book is not solely a study of the inheritance of the blood groups—it contains a good deal of serological information as well, and concludes with chapters on medico-legal applications and anthropological studies.

There is an introduction and then sections on the ABO, MNS, P, Rh and other blood-group systems. The main chapter in the book, however, concerns the Rh-blood group system, and of the 146 pages in the book, about 40 are devoted to this chapter. In it are described the details of the Rh–Hr system of notation and its various applications; anyone who is not familiar with Dr. Wiener's views on this subject will find them clearly set out in this chapter. Some of the other blood-group systems are rather sketchily dealt with, for example, the Lutheran, Duffy and Kidd systems occupying only 1–2 pages each, but in certain instances useful information is given in a condensed form; the Kell system is a good example of this. One of the less-pleasant features of the book is the use of bold-face type for certain of the blood group symbols; it would be better if this were discontinued. In certain of the rarer blood group systems, the authors substitute a notation of their own for that which is more widely and generally accepted by other workers. This, for example, is done in the Duffy and Kidd blood group systems; it is unfortunate that they have decided to do this.

This is a book summarizing many aspects of work on blood groups and will be of greater interest to those primarily concerned with blood groups than to the general reader.

F. Stratton

A Text-Book of Metallurgy

By Dr. A. R. Bailey. Second edition. Pp. x+561. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1960.) 30*s*. net.

THE first edition of this book, published in 1954, was very favourably received because it provided an elementary account embracing almost the whole field of metallurgy, from ore-dressing to the physical behaviour of metals, written in a concise and accurate manner. The general form of this book is retained in the new edition, but the author has made many additions referring to modern developments and has provided an adequate number of up-to-date references. The coverage of the book is so large that inevitably most subjects do not receive great depth of treatment and the level is that of first-year metallurgy at a university or the equivalent stages of a technical college course. It is a good preparation for the more specialized texts which must be used at a later stage. In any technological subject, a balance must be struck between basic theory and practice and indeed the author of the present book succeeds in blending these two aspects to form a harmonious whole. For example, the student can find in one chapter a simple treatment of dislocations, while in another he can read an admirable account of the various mechanical working processes used in industry. The book is, in the reviewer's opinion, the best of its kind available. It represents in these days remarkably good value for money, and students intending to read metallurgy have good reason to make it one of their first acquisitions.

Annual Review of Physical Chemistry

Vol. 10. Edited by H. Eyring, in association with C. J. Christensen and H. S. Johnston. Pp. viii+537. (Palo Alto, Calif.: Annual Reviews, Inc., 1959.) 7 dollars.

THE latest volume of this well-known publication includes nineteen chapters, fewer than usual but the chapters are longer. Each has a very full list of references, and a notable feature is the inclusion of much Russian literature. The authors are British, Norwegian and American. Besides familiar topics such as thermodynamic properties, electrolytes, heterogeneous equilibria and spectra, there are chapters on copolymers, proteins, nuclear and paramagnetic resonance, and high temperatures. Each chapter covers a wide field of literature; but in spite of detail and the necessarily brief treatment the book can be read with interest and should be intelligible to physical chemists generally. The authors have taken a good deal of trouble in this direction and have been very successful. It is impossible in a brief notice to indicate the contents of the chapters, but I found those on electrolytes and high temperatures of particular interest; other readers will certainly have different choices. This series of volumes should enable physical chemists to keep up to date in fields outside their own particular interests. The book is very well produced and the price is reasonable.

J. R. Partington

ROCKET AND SATELLITE INSTRUMENTATION

INSTRUMENTATION for rockets and satellites was the subject of a one-day symposium organized jointly by the British Interplanetary Society and the Society of Instrument Technology on September 1. The meeting was held at Manson House, London, and attended by more than one hundred members of the two societies and others. The morning session, at which Dr. W. R. Maxwell, vice-president of the British Interplanetary Society, took the chair, was devoted to papers and discussion of the instrumentation in the space-vehicle, while the afternoon session, under the chairmanship of Mr. C. C. Eltenton, president of the Society of Instrument Technology, was concerned with instrumentation at the ground stations.

The first paper, by Mr. M. O. Robins, of University College, London, formed a very appropriate introduction to the subject since it outlined the British space research programme as constituted in June of this year. In this context he defined space research to mean scientific research carried out by using the technique of rockets, either for vertical sounding of the atmosphere or for the launching of Earth satellites. Historically the British effort started with the first steps taken in the autumn of 1953 by the Gassiot Committee of the Royal Society which resulted in the development and launching of the *Skylark* rocket at Woomera in 1957. This reliable and versatile vehicle has enabled a variety of valuable scientific measurements to be made up to heights of 100 miles and more. These, carried out mainly by university groups, have included investigations of upper-atmosphere winds and temperatures, electron density, solar radiation and cosmic rays. Experiments planned for the near future include measurements of the geomagnetic field, observations on ultra-violet wave-lengths and ionospheric investigations using radio propagation methods.

The effort made in Britain to extract the maximum of scientific information from optical and radio observations of the Earth satellites launched by other countries had been very effective. The data obtained had already yielded new information about the ellipticity of the Earth, air density and the ionospheric regions. The positional information also enabled a satellite 'prediction service' for observers in the United Kingdom to be operated.

Turning to the British plans for the direct use of satellites, Mr. Robins spoke of the arrangements whereby British-designed instruments will be carried in the *Scout* satellites to be launched in the United States, and of the possibility that British satellites might be launched by a rocket system based on the *Blue Streak* and *Black Knight* designs. For the first British *Scout* payload, the main objective of the experimental programme will be the direct study of the electrical properties of the ionosphere. Present arrangements envisage three *Scout* payloads for the United Kingdom, and experiments for the second are being worked out.

Design studies of possible British satellites and of the investigations to be carried out in them are in progress. Three types of satellite are envisaged. The first, which would weigh about 1,750 lb. and go into a circular orbit of 300 miles height, could carry a telescope and spectrometer for ultra-violet astronomy of stars. The second, of 500 lb. in weight, might be capable of penetrating to distances of 10,000 miles and would enable studies to be made of the Earth's atmosphere, gravitational and magnetic fields. A third and smaller satellite capable of very elliptical orbits is also being considered.

The instrumentation for the first Anglo-American *Scout* satellite was described by Dr. A. P. Willmore, of University College, London. The satellite, which would be launched by a four-stage rocket using exclusively solid propellent motors, would be approximately 20 in. in diameter and would weigh in the region of 100–150 lb. Its orbital inclination would be above 50°, and its perigee and apogee heights about 200 and 600 miles respectively. The responsibility for the instrumentation would be divided between the U.S. National Aeronautics and Space Administration and the British universities; the former providing such parts as the telemetry and power supplies and the latter the scientific apparatus.

In all there will be six experiments on board the satellite: three ionospheric experiments designed to measure the density and temperature of the electrons and the composition of the positive ions, two to measure the intensity of radiation from the Sun in the ultra-violet and the X-ray parts of the spectrum, and in the last the primary cosmic-ray spectrum will be observed. The electron density in the ionosphere will be measured in two ways. In Prof. Sayers's experiment the capacitance of a parallel plate condenser immersed in the ionosphere will be measured at a frequency of a few megacycles per second, and from it the electron density calculated. In the method devised by University College, London, an electrode, consisting of a 2-cm. diameter disk mounted flush with the outer skin of the satellite, is maintained at a potential of 1 V. or so, negative with respect to the ionosphere. By varying this voltage sinusoidally the curvature of the voltage-current characteristic is determined and the density and temperature of the electrons deduced. The experiment to analyse the composition of the positively charged particles employs a similar technique using a spherical probe on a 12-in. long arm.

Ionization chambers and proportional X-ray counters will be used to monitor the emission of ultra-violet light, and soft X-rays from the Sun in order to correlate ionospheric storms with disturbances occurring in the atmosphere of the Sun.

Dr. J. Ring, of the University of Manchester, dealt in more detail with ultra-violet measurements from satellites. The main difficulties of ultra-violet measurement from the ground are due to absorption, scintillations and background radiation produced by the atmosphere and these could be overcome by satellite-borne instruments. There are, however, many difficulties in the design and operation of the instruments, the principal ones being alignment and the recovery of information gained. For example, the examination of a particular star by means of a spectrograph would necessitate alignment and follow-

ing to 10″, but, even if a resolving power of 1 Å. over a 2,000 Å. range were required, a band-width of only 100 c./s. would be needed for the transmission of the data. If, on the other hand, the intensity of the whole sky were to be measured at a given wave-length, then, although there was no alignment problem, the band-width of data transmission required would be many times greater. Dr. Ring described some of the work already done with rockets and satellites, and looked forward to future developments. It is planned to place into orbit an 8-in. telescope with television camera and also 36-in. and 50-in. telescopes. Although the first two objectives may be attained within three years it will probably be ten years before a 50-in. telescope is in orbit. He thought, however, that astronomical observations would really come into their own when manned satellites were launched.

The afternoon session was opened by Dr. B. G. Pressey, who described the ground equipment used for radio observations on satellites at the Radio Research Station of the Department of Scientific and Industrial Research. These observations had a three-fold purpose: to provide positional information, to obtain information from the instruments in the satellite and to study the propagation of the waves transmitted from the satellite. The instrument principally used for satellite tracking is the interferometer, in which the direction of arrival of the radio-wave is deduced from a measurement of the phase difference between the waves received on a pair of spaced aerials. Equipments for reception on 20, 40 and 108 Mc./s. are installed at the Radio Research Station, and the 'Minitrack' system, developed in the United States, which operates on frequencies 136–137 Mc./s., is now in course of erection on the same site.

The equipment available for the reception and analysis of satellite signals on frequencies from 20 Mc./s. to several hundred megacycles per second was described. It was the general practice to record the signals on magnetic tape—up to four channels simultaneously—and to analyse the records afterwards. Measurement of the Doppler frequency shift was carried out with the aid of a reference signal of high stability, the radio beat-note produced by mixing it with the satellite's signal being measured automatically on an electronic counter. The direct measurement of the difference between the frequency shifts on two signals on harmonically related frequencies was also described.

As an example of the methods of recording and decoding telemetry signals, the extraction of cosmic-ray data from the signals from *Sputnik* 3 was described. The signals, which consisted of a group of four pulses, were displayed on a cathode-ray tube using a linear time-base, and photographed on to a moving film so as to produce a raster type of record. From this record time intervals between the changes in pulse durations could be readily measured, and ray intensities and count-rates calculated. The method used to decode the more complex systems such as that used in *Explorer* 7 were also indicated.

A later paper by Messrs. D. J. McLauchlan and T. T. Walters, of the Bristol Aircraft Co., provided a detailed description of the 'Timtape' equipment developed for the processing of time-multiplexed telemetry data from test missile firings. The input is a magnetic tape derived from the Royal Aircraft Establishment 24-channel telemetry system. After separating the channels the equipment interprets the data relative to the calibration-levels and converts them from analogue to digital form. The output is in the form of photographic records and punched cards. Although designed specifically for one telemetry system, 'Timtape' should be capable of reducing data from other systems without extensive modification.

The remaining paper differed from the others in that it dealt with rocket propulsion instrumentation. In presenting it Mr. A. W. T. Mottram, of Bristol Siddeley Engines, Ltd., reviewed the techniques used for the measurement of the performance of liquid-propellent rocket engines. The most important quantities to be measured are thrust and propellent-flow, and these are required to a high degree of accuracy. Measurement of system pressures, flows and thrust under fluctuating conditions demand less accuracy since their purpose is limited to the establishment of engine behaviour and the qualitative assessment of system defects. The prime requirement is for reliability, for which reason the simplest type of instruments were recommended. The best basic-thrust measuring transducer is a rubber-bonded hydraulic capsule with a pressure transducer of the unbonded-wire strain-gauge type. A free turbine flow-meter had proved to be the best for determination of propellent-flow, and Bourdon gauges were recommended for the measurement of pressure.

The symposium provided a useful review of the instrumentation used in satellites and on the ground for investigations of the upper atmosphere. The full texts of the six papers, together with the discussions on them, will be published in the *Journal of the British Interplanetary Society*.

B. G. Pressey

SPECTRUM OF RADIATION BACKGROUND UNDER WATER

By GORDON K. RIEL, JOHN P. DOLFIS and DONALD G. SIMONS

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THE investigation of physical phenomena occurring in the sea has, in many cases, lagged behind similar investigations on land and in the atmosphere. This is certainly true in the field of radiations from cosmic rays and other naturally occurring sources. A detailed knowledge of these radiation backgrounds is necessary to evaluate any underwater programme in which detection of radiation is required. An analysis of the radiation background is especially important, because, while the sea provides some shielding from cosmic radiation, it contains several radioactive nuclides. This work has an added importance since any programme of measurement of radiation which is to be carried out in the sea will be

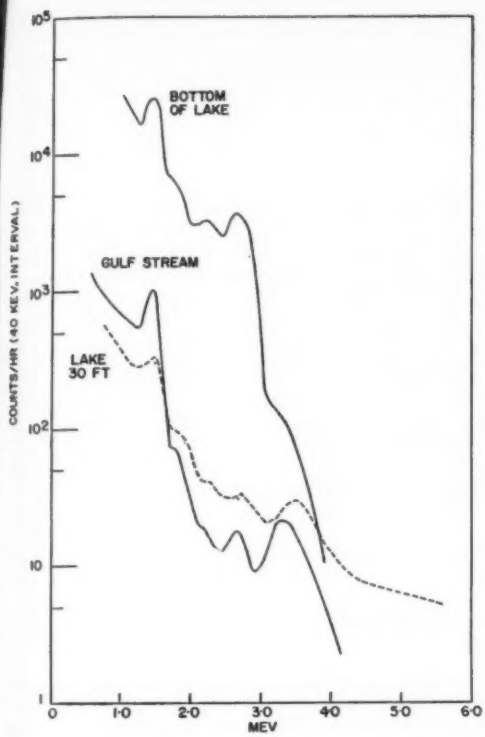

Fig. 1. Spectrum of natural radiation measured under water

almost invariably of a low-level nature, thereby requiring an extensive knowledge of the background sources present and their intensities. Such low-level counting programmes may include: certain types of nuclear research problems which require low cosmic-ray backgrounds, tracer studies of ocean currents, tracer measurements of corrosion or leakage, measurements of the concentration of radioactive nuclides in sediments and biological specimens, and measurements of the effectiveness of radioactive waste disposal.

Hitherto, the problem of obtaining such information in the sea has been made difficult because of its relative inaccessibility to specialized nuclear instrumentation. Consequently, radiation measurements involving energy analysis were limited to laboratory examinations of samples taken from the sea. These methods, while instructive and useful for particular problems (for example, backgrounds from biological specimens), were inherently unable to present a true picture of the background radiations under the surface of the sea. The recent availability of transistorized, light-weight, multi-channel analysers, however, has made it possible to conduct spectral measurements of γ-radiation and cosmic radiations *in situ*.

The experimental phase of a programme to measure the energy spectrum of background radiation directly in the sea was begun in January 1960. The results presented here are from these tests and represent, to our knowledge, the first such direct measurements of the naturally occurring radiation backgrounds. A scintillation spectrometer consisting of a 5-in. diameter by 6-in. long sodium iodide (thallium) crystal, and a transistorized 128-channel analyser for pulse-height sorting, was used for this test. The sodium iodide crystal and associated electronics were encased in a sealed probe and lowered from a surface ship by means of cables up to 1,000 ft. long. A single-conductor armoured cable was used to supply voltage to the probe and return the signal to the ship. On board ship the 128-channel analyser recorded digitally the energy spectrum within a pre-selected range.

Fig. 1 shows the energy spectrum of radiation measured under the water. The curve labelled 'Gulf Stream' gives the cumulative results of two tests at a depth of approximately 400 ft., and a third test at about 1,000 ft. The total counting time of these three tests is 21 hr. These tests were conducted in the Gulf Stream near Fort Lauderdale, Florida. Included for comparison are measurements which were made at mid-depth and in the bottom sediment of the fresh-water of Tridelphia Lake, Maryland. Although the surface background is not shown, it was similar in distribution and magnitude to that found in the bottom of the lake. The general trends of the backgrounds obtained in the sea and lake are lower by an order of magnitude than that at the Earth's surface. This, of course, is to be expected because of the shielding that the water furnishes from cosmic-ray events and from naturally occurring radioactive chains.

The spectral results show essentially three major energy peaks at 1·46, 2·6, and 3·3 MeV. and two minor peaks at 1·8 and 2·2 MeV. These energies are present in both the fresh- and sea-water. The most prominent peak is that of 1·46 MeV., which is due to potassium-40. This is higher in magnitude in the sea than in fresh-water. (No correction has been made for the potassium-40 content of the detector probe itself.) The higher activity in the salt-water is due to the fact that potassium salts, being mostly soluble, concentrate in the ocean. It should also be noted that potassium-40 gives the major background contribution of all the radiation sources present by more than one order of magnitude. Thus, if no energy discrimination is used, the radiation detector will be sensitive almost exclusively to changes in the concentration of potassium-40. Below 1·46 MeV., the scattered potassium-40 radiation masks all other sources.

The peak at 2·6 MeV. corresponds to the 2·61 MeV. γ-ray from thallium-208 in the natural thorium-232 chain. Since these elements are primarily present in compounds of very slight solubility, they concentrate in the bottom sediment. Furthermore, some activity can be expected in the shallow lake water due to mixing of the sediment and washing of soils into the lake by ground run-off. Thus, in addition to the higher cosmic-ray background in the shallow water which tends to increase the rate of count throughout the entire spectrum, a further increase in activity in the 2·6 MeV. region was expected in the lake and at lake bottom. This was clearly demonstrated when the probe was embedded 18 in. into the sediment of the lake bottom. Even though the amplitudes of all of the peaks mentioned were increased in the sediment as compared with the water, the increase of the 2·6, 2·2, and 1·8 MeV. radiations from the heavy elements is an order of magnitude larger than that of the 1·46 and 3·3 MeV. radiations.

The small break at 2·2 MeV. is probably due to the 2·2-MeV. radiation of bismuth-214 from the natural uranium-238 chain or bismuth-212 from the thorium-232 chain. Similarly, the break at 1·8 MeV. may be

due to the 1·8-MeV. γ-rays from bismuth-212. These breaks are also due, in part, to the Compton edges from the 3·3- and 2·6-MeV. peaks respectively.

The exact source of the prominent peak at 3·3 MeV. is not known at the present time. Laboratory tests have shown that this radiation exists as a contaminant of the steel pressure vessel itself, and is not a true source present in the water. Measurements are now in progress to check this further and to try to identify this source of radiation.

Events at energies higher than those originating from the naturally occurring isotopes are assumed to be of cosmic-ray origin. The energy spectrum up to 9 MeV. was measured in the Gulf Stream. There were no peaks from 4 to 9 MeV., and the relationship of count-rate to energy as obtained from the spectra in this range can be represented empirically by :

$$N_E = 54 \exp - 0{\cdot}72E$$

where N_E is counts per hr. in a 40-keV. interval, and E is energy in mega electron-volts.

In the Gulf Stream, the count-rate above 8 MeV. was 240 counts per hr. with 400 ft. of cable, and 70 counts per hr. with 1,000 ft. of cable. Note that the length of cable is quoted rather than depth, since no accurate measurement of depth was available. In the lake, the count-rate above 6 MeV. from 10 to 50 ft. in depth can be fitted by the following function :

$$N_D = 220 \exp - 0{\cdot}21D$$

where N_D is counts per minute at depth D in feet.

One difficulty in measuring γ-ray spectra under water is that the radiation is partially degraded in energy by scattering in the water. This tends to smear out the radiation spectrum. Therefore, the features of the resulting spectrum are not as sharp as would be obtained with non-distributed sources. The unfavourable geometry makes estimation of the concentration of radioactive nuclides difficult. A new probe designed to improve the counting geometry is under construction. It will be capable of exploring the radiation at depths in the seas beyond the practical reach of cable support. This will be achieved by lowering the new probe with a free-diving vehicle. Further tests are also planned using the present equipment.

THE SENSE OF HEARING

IT is probably true to say that the eye has received, and continues to receive, from both physiologists and psychologists more attention than has the ear. Nevertheless, speech is one of the most important characteristics of human beings, and the sense-organ with which it is heard deserves therefore to be given due honour. A session of Section I (Physiology) of the British Association, held in Cardiff on September 2, took hearing as its central topic, and left no doubt that this sense is as fascinating as any other.

The main feature of the session was the presidential address to the Section by Prof. W. Burns (Charing Cross Hospital Medical School). He considered the response of the ear both to normal and to intense sound, starting by describing the extraordinary sensitivity and flexibility of the organ. It can detect pressure changes in the atmosphere so small that the actual receptor cells are moved through only the diameter of a hydrogen atom, so that any further increase in sensitivity would merely result in the Brownian movement of the air becoming audible ; and yet the ear can also operate for short periods at pressure-levels a million times greater. Part of the secret of this extraordinary performance lies in the mechanical linkage which transmits the sound from the ear-drum to the inner ear where the sense-organs themselves are. The bones which form this linkage terminate in a small piston having only one-twentieth of the area of the ear-drum, and this piston therefore transfers a considerable pressure per unit area to the fluid inside the closed container of the inner ear. In addition to this aid to sensitivity, some protection against loud sounds is provided by the mechanical linkage since muscular contractions can increase its resistance to movement when such sounds are heard and so reduce the sound-level actually reaching the sense organs.

The receptors themselves are arranged, as has long been known, along the basilar membrane which lies inside the coiled tube known as the cochlea. The basilar membrane runs from end to end of this tube, being narrow at the end where the sound enters and wide at the far end : it was of course suggested at one time that it resembled a set of piano-wires resonating independently to different frequencies of sound, but this does not fully correspond to modern ideas. It would seem rather that a change in pressure at the entrance to the cochlea sets up a travelling wave in the basilar membrane which starts at the narrow end, sweeps up towards the far end, getting larger as it goes, and then dies away. The point at which the greatest displacement of the membrane occurs depends on the mechanical properties of the system and thus varies for different frequencies of stimulation. In general, high frequencies mainly affect the narrow end of the membrane while low frequencies involve the entire length.

The sense-organs themselves are cells about the size of a red blood corpuscle, which rest on the basilar membrane with small bristle-like projections (hairs) sticking up into another membrane, the tectorial membrane, which forms a second 'shelf' above the basilar membrane. The displacement of these membranes causes the hairs to be bent. Now there is a potential of about 150 mV. between the inside of the cells and the fluid in which the hairs terminate. The size of this endocochlear potential depends on the extent to which the hairs are bent at the time, and alternating movements of the membranes will therefore produce an alternating potential, the cochlear microphonic, which can be picked up by electrodes in a number of places in the ear. The microphonic follows sound stimulation very closely with no sign of the all-or-none characteristics of the sensory nerve, and each negative phase of the microphonic is followed by the occurrence of nerve impulses in the auditory nerve. It seems likely therefore that the electrical energy stored in the hair cells somehow

produces the firing of the nerve fibres, although the link between the two is still unknown. The stored electrical energy thus acts as a kind of biological amplifier, triggering off considerable effects from a small input of sound energy.

The message in the auditory nerve, then, carries information about the frequency and the intensity of the sound. Different frequencies of sound give rise to maximum stimulation of different hair-cells and therefore of different nerve fibres: at least this is so at frequencies above 2,000 c./s. or so. Below that frequency each wave of the stimulus gives rise to a burst of impulses in the nerve, and the frequency of the impulses therefore conveys information about the frequency of the stimulus. The higher the intensity of the sound, the greater the number of fibres which become active in the auditory nerve: and, at high frequencies of stimulation, intensity will also be reflected in an increased frequency of firing of the fibres.

Perhaps the most important practical implications of Prof. Burns's paper arose from the question of effects of noise on the ear. On the basis of present knowledge, he suggested that special steps must be taken to preserve the hearing of industrial workers if they are exposed throughout their working life to noise above particular levels. The noise should be analysed into octave bands, and protection begun if the sound pressure-level in the octave below 4,800 exceeded 80 db. above the usual arbitrary zero of 0·0002 dyne/cm. The corresponding criteria for successive octaves down the spectrum were 80, 85, 85, and 90 db. If these minimum levels were exceeded, steps should be taken to reduce the exposure of the workers, and to check their hearing regularly by audiometry. Since occupational loss of hearing tends to appear first at 4,000 c.p.s. and spreads only later to lower frequencies, it can be detected and the workers moved to safer employment before the deafness becomes too damaging socially. This is the surest way of detecting the most susceptible people, although it is being widely assumed that the amount of temporary deafness produced by a short experimental exposure to noise may predict the susceptibility to more permanent deafness after long exposure. Above all, it must be realized that occupational deafness is insidious and need not be noticed by the victim until well advanced: nor is it connected with annoyance, being just as dangerous to people who do not object to noise, and conversely being no hazard at the levels reached by many annoying everyday sounds.

Mr. D. E. Broadbent (Applied Psychology Unit of the Medical Research Council) took up the question of noise, and considered those of its effects which do not involve the ear except as the point of entry of the noise. Many of the alarmist reports often spread about these effects are not supported by the evidence: large-scale studies by the U.S. Navy have failed to find any effect on mental health from noise, nor is there much support for the idea that metabolism is higher when working in noise. The sexual performance of rats remains normal after exposure to very high levels, and although the adrenals both of rats and men appear to be active at such levels, there is no evidence of pathological changes as a result. Some recent German researches do suggest a possible effect on the peripheral blood vessels, which constrict in noise, but the chronic effects of this response need further investigation. In addition, of course, there are widespread changes in the body at the onset of an unfamiliar noise, as there are in any new situation. These changes (increased pulse-rate, lowered skin conductance, etc.) are really a response to novelty, and cannot be used as arguments against noise without putting in jeopardy other stimuli which have the same effect: for example, meeting a strange member of the opposite sex.

The efficiency of mental work is definitely impaired by noises of more than 90 db., but has not been proved to be so by any fainter noise. The effect takes the form of momentary lapses, between which work may be perfectly normal. Mr. Broadbent was particularly concerned to make a distinction between these relatively slight effects on health and efficiency, and the annoyance caused by noise. The latter was very widespread and natural, even though it had no connexion with the former: even animals try to avoid loud noises, and sounds which they associate with pain; and to a behaviourist one object of civilization is to decrease the frequency of those stimuli which are avoided by human beings. Annoyance is thus an adequate reason for noise abatement, and the cause of the latter can only be harmed by exaggerated claims of effects on health and efficiency.

Dr. T. S. Littler (Wernher Research Unit of the Medical Research Council) then spoke about problems in the testing of hearing (audiometry). Since any complex periodic wave can be regarded as the sum of a number of sine waves, and since the ear does to some extent analyse complex sound in this way, the most developed method of testing hearing is to present to the ear sine waves of various frequencies, and determine the lowest intensity at which they are audible. Since the loudness and pitch of short tones vary with their duration, the test tone must be long enough to avoid these distorting effects: furthermore it must start and stop gradually rather than abruptly, since sharp onsets are heard as clicks and do indeed contain frequencies widely different from that intended. The whole test tone therefore, including its gradual beginning and end, should last at least two seconds.

The intensity of a threshold tone can be specified either as the minimum audible pressure or the minimum audible field: the former term means that the sound was delivered by head-phones and the sound pressure-level, produced by the head-phones, measured. The minimum audible field means that the sound was produced by loudspeakers and the level measured in the position where the listener's head was later to be placed. The two measurements will not usually be the same, partly because of the physical differences in the situations, and partly because of the sound of the listener's own pulse and similar disturbances which are more notable with head-phones.

There will not, of course, be found a definite intensity at which sounds change from inaudible to audible: over a certain range, the proportion of test tones heard will increase as the sound pressure rises, and the threshold is best taken as the level at which 50 per cent of test tones are heard. This level can then be compared with that for normal ears: it has been difficult to get adequate measurements of normality, because samples of the general population are liable to include people with slight undetected hearing loss. But recent measurements at the National Physical Laboratory, and by the Royal Air Force, have provided an adequate reference against which any given person can now be compared.

To some extent deafness due to failure in the mechanical linkage of the middle ear can be distinguished from deafness due to damage to the sense-

organs themselves or later stages in the nervous system. This is because audiometry by bone conduction will show no loss in the case of damage to the middle ear. But bone conduction is less well standardized than air conduction. The same may be said of speech audiometry, in which words are presented for identification at various sound pressure-levels: in this case it may in some people be impossible to obtain perfect performance even at levels well above the threshold, and it is clear that the performance of the ear at supra-threshold-levels needs further investigation.

The tenor of the whole session was summed up by Lord Adrian, who, in the discussion, reminded the meeting of Darwin's difficulties in explaining the eye on an evolutionary basis because of its complexity. The ear may well inspire similar feelings of admiration.

D. E. Broadbent

EDUCATION OF SCIENTISTS

THE respective roles of the universities, colleges of advanced technology, research institutes, and other institutes of higher education in promoting the education of scientists were extensively considered at a meeting of Section L (Education) of the British Association for the Advancement of Science on September 1.

Prof. M. W. Thring dealt with the role of the universities in teaching technology. He emphasized that technology has two aspects: that of applied science, which aims at presenting methods of working out rough-and-ready design formulæ based on first principles, and engineering, which is concerned with accurate design based on more empirical formulæ. The essential difference between pure and applied science is in the aim, and not in the quality of thought or level of fundamentality, the aim of pure science being essentially inquisitiveness, and that of applied science the benefit of mankind. The fundamental aspect of applied science is the development of the coefficients in the differential equations describing the separate parts of the complex processes that occur in industry. The superficial, or descriptive level of applied science is the *ad hoc* solution of practical problems. The corresponding level of pure science is natural history, empiricism, or classification, while the more fundamental aspect of pure science is the *experimentum crucis* to decide between rival theories. Since the only valid 'snobbishness' is that relating to quality of thought, it is unfortunate that many schools have inherited the older idea that there is a 'snobbishness' between pure and applied science. The basic problem is to relate the choice of subject of the students of university standard to the careers which are available for them when they have graduated. There is a great tendency for a boy to choose a subject that he has learned at school, or one which his master studied at university, and since very few applied scientists go in for teaching, this does not produce a correct balance in the choice of careers.

The introduction in Britain of colleges of advanced technology improves the channels leading to careers in applied science, but does not help to improve the proportion of the stream flowing in these channels. The essential problem is thus to show the brightest schoolboys that applied science is worthy of their talents and can give them fascinating careers, and is not just a place for those boys who are not deemed good enough for pure science by their schoolmasters. We need many more brilliant technologists on the boards of industry to give a new look to industry. Prof. Thring said that he has direct personal knowledge of industries which rely on foreign countries for their development work, and always wait until processes have been developed abroad before they take them up in Great Britain. The reason for this is lack of sufficiently brilliant technologists on the boards of the companies concerned.

The increased numbers of boys going to universities in the next decade, which would correspond to 5–6 per cent of any age-group, meant that one has to distinguish clearly between the static and dynamic view of university teaching. The former looks on students as a series of pebbles of predetermined size, and the function of the university is to sieve the larger ones and separate them rigidly from the smaller ones. Such a view does not, however, correspond to the human possibilities of the students concerned. The dynamic view is that all the 5–6 per cent of the age-group are capable of striking sparks of originality and interest, providing the teaching method can bring them out sufficiently. Somehow it is necessary to show the students that both the fundamental scientific laws and the way in which they explain practical processes have excitement and even glamour. So often the choice of a field of work for a student arises quite accidentally because one part of science has a good teacher, when many other branches of applied science could be presented in an equally exciting way. Some parts of applied science are inherently glamorous, such as nuclear fission, electron theory and rocket combustion; other parts require much more careful handling before the student can see the excitement of these subjects. The blackboard and chalk are no longer the only method of arousing the interest of the student. Colour films, television programmes, working models and working diagrams have all to be pressed into service.

In the Engineering Faculty at the University of Sheffield it has been decided that the size of the classes should by all attempts be kept below 50 so as to retain the close personal contact between the lecturer and the students. With larger classes one might just as well have a colour film of a lecture given once and for all by a national authority. Other points which are very important in the dynamic view of university teaching are the introduction of small supervision groups of not more than two or three students for an hour a week, keeping the number of hours of formal instruction as low as possible—the figure of 30 for the absolute maximum is being suggested—and the introduction of research or design-study problems in the final undergraduate year.

Prof. Thring then said that just as industry must have two legs, the production of goods for sale and

the development of new processes and methods to be a viable organization, so a university department must have teaching and research if the teaching is to be at the best possible standard. Hence, the university applied science department must have a strong research school studying the coefficients in the differential equations of the processes that are of interest in applied science, and this teaches the research students to be self-propelling in research. Prof. Thring confirmed that the civic universities are proud of their efforts to make sure that technologists know what is going on in the humanities, but felt that there should be no compulsion in this matter. He regarded the basic unit as the informal discussion group or the coffee party. In the discussion it was agreed that there is a difference between technology and the needs of industry for applied scientists among school-teachers, and various methods of overcoming this were discussed.

Dr. J. S. Tait, of the Northampton College of Advanced Technology, then discussed the role of the colleges of advanced technology in the education of scientists. In the 1890's a number of colleges of technology were formed as a result of Lord Kelvin's views that the universities were not close enough to industry, and those in London were particularly fortunate in that later on they could prepare students for the internal degree of the University of London.

By 1956 the Government decided that it would be sensible to give a small number of selected colleges of acknowledged reputation the opportunity to develop into colleges of advanced technology and discard all their work below university-level. They were to concentrate on producing the high-quality technologists so badly needed by British industry and research organizations. The colleges of advanced technology aim to produce the leaders of to-morrow by giving a good grounding in the applications of science to engineering. The new award of the Dip. Tech. is intended to have the standing of a second-class honours degree, and the colleges of advanced technology are equivalent to the technical universities of the Continent. The Committee of Vice-Chancellors and Principals of the Universities has recommended that holders of the Dip. Tech. should be regarded as eligible for university higher degree courses. The official entry conditions are six subjects in the General Certificate of Education, of which two must be at Advanced Level for grammar school boys, or better than 50 per cent in the Ordinary National Certificate for boys from industry, but of the 700 students taken so far in Dr. Tait's College, more than half have had three Advanced Level subjects at entry, that is to say, they have the minimum university entry requirements. Two-thirds of the total have been grammar school boys, and approximately 15 per cent public school boys. The wastage has only been about 5 per cent a year. The selection has been very severe since industry is paying about £2,000 per student to provide what, in effect, are scholarships without a means test. The student spends from six months to a year in industry before he starts in the college, and this makes a tremendous difference in his mental outlook, giving him a definite aim for his academic studies. The interest of the students in the humanities is fostered by giving them a 2-hr. lunch period during which lectures in the humanities are available. It was emphasized on behalf of the Department of Scientific and Industrial Research that it is prepared to give post-graduate studentships for work at the colleges of advanced technology, but the only qualification which can be given in this case is membership of the College of Technologists, while there is a tendency among students to feel that the Ph.D. is the only postgraduate qualification to which it is worth devoting two or three years of their lives. Prof. Craig pointed out that the technical universities on the Continent have their students for five and a half years full-time, and he emphasized that there were essential differences between the aims of the colleges of advanced technology and those of the universities, both being concerned with getting fundamental ideas across to the student, but differing in depth and generality.

Sir Patrick Linstead reported on the work of the British Association Committee, of which he has been chairman, on the subject of broadening the education of the graduate scientist. The desire for knowledge has three aspects: the desire to know how things work, the need to open the doors to a professional career in subjects in which the student is vitally interested, and thirdly, the need to be a member of the world and play his full part in society. On one hand, the student is exposed to lectures on outside subjects and told how important these are; on the other hand, the need to concentrate on his specialist subjects if he is to get through his examinations is emphasized to him. The scientist cannot be complaisant if he does not know of the humanities because of the increasing importance of scientists in the world. Inventiveness and scientific wisdom can only be supplied by vocationally trained scientists, but they also need a wider training as more and more of them are rising to top administrative positions.

The British Association Committee has concluded that both schools and universities have important parts to play in widening education beyond the formal teaching. The schools require more time for minority subjects in the sixth form. In the universities he agreed that compulsion was undesirable, but four steps were necessary: first, the reduction in the pressure on the main specialist subject; secondly, greater attention to non-scientific subjects which are, in fact, a necessary part of the specialist subjects, such as the use of English and the introduction of the history of science; thirdly, the provision of cultural events, lectures, concerts and library; fourthly, the generous provision of facilities, libraries of cultural books which do not close at 5.30 p.m., halls of residence with coffee bars, and beautiful surroundings. Sir Patrick also discussed the question of full-time postgraduate courses, which he regarded as very important. He directed attention to the fact that it would be necessary to have relatively small numbers in such courses compared with the undergraduate courses, while the elaboration of materials and lectures was very great. It was therefore essential not to have excessive competition between different teaching institutions, but to distribute the subjects between them. Prof. Craig also wanted more postgraduate courses, both specialized in advanced technology with people returning from industry, and as extensions of undergraduate courses to more advanced mathematical methods and fundamentals. These should be immediately after the undergraduate course. Prof. Craig also felt that the history of science should be taught with day-to-day reference to the subject as it occurred, and not starting from the very ancient beginnings of the science.

M. W. Thring

HORMONES AND THE CONTROL OF ROOT GROWTH*

By Prof. H. E. STREET
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THE aspect of the hormonal control of growth which has been most intensively studied is the expansion phase in the growth of shoot cells. The promotion of this phase of cell growth is a characteristic activity of indolylacetic acid, the only auxin which has been isolated from plants and is known to be widely distributed. This emphasis is reflected in the definition[1] of auxins as "those plant growth substances which are characterized by their property of stimulating extension growth of shoot cells in bioassay tests". Current texts of plant physiology have, in consequence, very little indeed to say regarding the natural growth hormones of roots and their role in controlling the growth and differentiation of root cells.

It was from the early studies of the influence of the root tip upon the positive geotropic sensitivity of roots, initiated by Cholodny[2,3], that there developed the widely accepted view that the root tip produces an auxin similar to, or identical with, that secreted by the coleoptile of grasses and that the concentration of auxin thereby established in the root cells is supra-optimal for root growth. The subsequent demonstrations of the presence in roots of substances active in the *Avena* curvature test[4] and their production by isolated root tips, particularly when these are supplied with sugar, supported this view. A number of workers[5–7] did, however, report that externally applied auxins are by no means always inhibitory to the growth of seedling roots, and more recently the careful decapitation experiments of Younis[8] have shown that the removal of the root meristem does not lead to an acceleration of root extension growth although it does, as earlier claimed, reduce the sensitivity of the root to gravity.

New approaches to the study of the hormonal control of root growth have, however, now developed from the application of paper chromatography to the separation of natural growth-active substances[9,10] and from studies of the growth of excised roots in sterile culture.

Chromatography has been used to study the natural occurrence in roots of both auxins[9–15] and substances with gibberellin activity[16]. The chromatographic solvents used have in the former case been those known to effect separation of indoles, in the latter case to effect separation of gibberellins from naturally occurring inhibitors. Most of the auxin studies have the limitation that the chromatograms have been assayed only by the *Avena* straight-growth test[17]; directed, that is, to the detection of auxins as defined by their activity in promoting expansion of shoot cells. The simultaneous use of pea-root segments[11] or excised root cultures (Winter, unpublished) as test objects should increase the value of such surveys when working with root extracts.

There is a measure of agreement between different laboratories as to the pattern of auxin activity found on chromatograms of the 'ether-soluble' fraction yielded by roots[11,12,15]. The work of Thurman and Street[15] reveals activity at the R_F of the "accelerator α" of Bennet-Clark and Kefford[10,11] and of indolylacetic acid; elution and rechromatography of the "indolylacetic acid zone" in various solvents strongly indicates the presence of indolylacetic acid. At a higher R_F there is an acidic inhibitory zone which corresponds, with *iso*propanol/ammonia/water as solvent, to the "β-inhibitor" of Bennet-Clark and Kefford. The chemical nature of both the α and β zones is, however, obscure. Finally, there is activity at the solvent front which, when rechromatographed in 40 per cent ethanol, is shown to be complex and apparently does not contain indolylacetonitrile in detectable amount. These chromatograms of the preferentially 'ether-soluble' constituents of root extracts have so far only given negative results when treated with various indole and other sensitive spray reagents, which may indicate that the active compounds are present in very small amounts.

When the alcohol-soluble material of roots in aqueous solution is exhaustively extracted with ethyl ether or ethylacetate significant auxin activity remains in the aqueous phase. This 'aqueous' fraction has been submitted to chromatography and bioassay in several laboratories[13–15], but the published chromatograms do not reveal a consistent pattern, nor have the discrepancies been satisfactorily explained. Thurman and Street[15], by adopting certain purification procedures, combined with low levels of chromatogram loading as compared with those required for the detection of activity in 'ether' fractions, have demonstrated an indole and ninhydrin reactive zone. Part of the growth-promoting activity of this zone is attributable to the indolic amino-acid, tryptophan, and part to other unidentified ninhydrin-positive substances. The 'aqueous' fraction also yields, at a higher R_F in *iso*propanol/ammonia/water, a broad zone of growth-promoting activity resolvable by rechromatography into discrete growth-promoting ninhydrin-positive zones which do not correspond in R_F with any of the commoner amino-acids. Sugars detected on these chromatograms could be separated from the growth-active zones.

The purpose of describing these results in some detail is to direct attention (1) to the complexity of the picture in these and other studies involving chromatography, (2) to the evidence for the presence of indolylacetic acid albeit at very low concentration, and (3) to the quantitative importance of the preferentially water-soluble constituents, including tryptophan, in the total 'auxin' activity extractable from root cells. To this must be added the recent demonstration by Butcher[16] that tomato roots also contain substances with gibberellin activity as revealed by the dwarf pea assay[18].

The complexity of the indole system in roots is further suggested when one examines by chromato-

* Substance of a paper given before Section K (Botany), at the Cardiff meeting of the British Association on September 7.

graphy (even at low levels of loading) the 'ether' fraction obtained from roots which have been allowed to take up indoles from the external medium. These indoles have, in unpublished experiments carried out in my laboratory by Thurman, been applied at inhibitory but sufficiently low concentrations that the roots can recover from the experimental treatments as shown both by subsequent root growth and depletion of the accumulated compounds on transfer to indole-free medium.

Feeding with indolylacetic acid (10^{-6} gm./ml. for 24 hr.) leads to a marked enhancement of growth-promoting activity at the indolylacetic acid position, to the appearance of Erhlich and nitric–nitrite reactors and to growth-promoting activity at R_F 0·25 (*iso*propanol/ammonia/water). The two strong nitric–nitrite reactors at R_F 0·25 and 0·35 in this solvent, can be resolved into three distinct reactive spots by rechromatography in butanol/pyridine/water. One of these has been shown by hydrolysis to be indolylacetylaspartate, the second corresponds in R_F with indolylacetylglutamate and the third the nature of which is unknown gives a characteristic pink colour with nitric–nitrite reagent (the same compound seems to have been detected by Andreae and van Ysselstein[19] in stem tissue). In addition, the *iso*propanol/ammonia/water chromatograms showed two other indoles, one at the starting line and the second at the R_F of indolylacetamide.

Feeding with indolylacetonitrile (10^{-5} gm./ml. for 24 hr.) gives high growth-promoting activity at the indolylacetonitrile position, but the only other compound detected was inactive in the *Avena* test and corresponded in R_F and Ehrlich reaction to indolylcarboxylic acid. This suggests the operation here, as in some other plant tissues[20], of an α-oxidation of the nitrile via indolylaldehyde without any intermediate formation of indolylacetic acid. Feeding with tryptophan (50 mgm./l. for 24 hr.) leads to the marked accumulation of this amino-acid, but the 'ether' fraction lacks growth-promoting activity (at the low level of loading adopted) and the only ethylacetate-soluble compound detected is probably the malonyltryptophan, previously detected in tomato tissue, both as a natural constituent and as a product of tryptophan metabolism, by Good and Andreae[21].

Two interesting observations may be noted from these feeding experiments. First, indolylacetonitrile feeding does not enhance activity at the indolylacetic acid position or give the Ehrlich and nitric–nitrite reactors associated with indolylacetic acid feeding. Further, when indolylacetic acid and indolylacetonitrile are fed simultaneously to roots, the resulting chromatogram is equivalent to an indolylacetonitrile chromatogram superimposed upon an indolylacetic acid chromatogram. This suggests that if there is any inhibitory effect of indolylacetonitrile on indolylacetic acid metabolism, as reported by Bennet-Clark and Wheeler[22] working with disks of potato tuber tissue, then this is quantitatively unimportant in the root tissue. These new observations, when considered in the light of earlier published physiological studies with indolylacetic acid and indolylacetonitrile[23,24], strongly support the view that the activity of indolylacetonitrile on root growth is not explicable in terms of its acting as a precursor of indolylacetic acid. The second interesting observation is that the relatively high levels of indolylacetic acid and indolylacetonitrile and of the indolylacetic acid–amino acid conjugates established in these feeding experiments rapidly decline on transference of the roots to indole-free medium, suggesting their rapid further metabolism. It should be emphasized that these feeding experiments were only designed to detect major ether-soluble products arising from the absorbed indoles and would not detect protein-indole complexes reported by Seigel and Galston[25] to arise from feeding auxins to pea root apices.

As already indicated, 'active' compounds detected in root extracts have been so defined primarily by reference to their ability to promote the expansion of shoot cells. Clearly, therefore, it is important to study the effects of known plant hormones and chemically related compounds on root growth. Using root cultures growing under sterile and defined nutritional and environmental conditions the physiological activity of such substances can be readily described in terms of root morphology, cell division, cell expansion and tissue differentiation. Comprehensive studies of this kind have already been initiated with indolylacetic acid and indolyacetonitrile and revealed their contrasted effects on cell division and cell expansion[23,24].

One of the problems raised by such studies was that auxins seemed to be, at sufficiently low concentrations, inactive, and at higher concentrations only inhibitory to root growth; a situation which took us back to the Cholodny hypothesis that auxins function only as inhibitors of root growth. Recent work[26,27] has, however, shown that appropriate manipulation of the nutritional conditions of culture, particularly in respect of the carbohydrate status, enables significant enhancements of root growth to be obtained in response to treatments with auxins and also with gibberellins and kinetin. Further, since the standard conditions of root culture have been worked out in relation to a very few species, in fact primarily from work with certain tomato root clones, it is not surprising that the bringing into culture of the roots of new species, under probably sub-optimal conditions, has also served to show that very marked stimulations of root growth can be obtained from the applications of low concentrations of auxins and related compounds[28,29].

Work involving the application of mixtures of auxins, gibberellins and kinetin to root cultures has shown that the rate of growth, the relative contributions of cell division, expansion and differentiation to growth, the degree of apical dominance and so on are, under certain conditions, determined less by the concentration of any one factor than by the balance between them[16]. Comparative studies between species of the factors controlling the persistence of meristematic activity in root apices also point to the operation of more than one hormonal factor[30]. These physiological results, when considered against the background of the complex picture revealed by chromatography, suggest that in root growth and differentiation the balance between a complex of growth factors may be determinative in a way similar to that so dramatically exposed by Skoog *et al.* in their studies on growth and differentiation in shoot callus cultures[31].

Recently, in work with cultures of cereal roots, there has been obtained evidence of a further aspect of the hormonal control of root growth. Roots of cereals have, in general, proved resistant to culture[32]. Root tips excised from seedlings, when transferred to standard root culture media, show initially a high growth-rate, but this declines rapidly and ceases within 2–3 weeks. Subculture of the root tips enhances this decline and clones cannot be established in culture.

However, Roberts and Street[28] succeeded in establishing clones of excised Petkus II rye roots by incorporating tryptophan into the culture medium. Further, the observation that, in this case, D- and L-tryptophan and indolylacetic acid were all equally effective suggested that the active substance was not tryptophan itself but some compound for which it acted as a precursor. This view was strengthened when it was found that tryptophan samples, if incorporated aseptically and without heating, were either inactive or of very low activity and that activation occurred during autoclaving. Rye roots apparently ceased growth, in the absence of hormonal concentrations of a tryptophan derivative, for lack of some essential growth hormone.

In unpublished experiments, Scott, Carter and Sutton have now studied the growth in culture of excised roots of the Atson variety of wheat and found that the level of growth and its persistence can be dramatically enhanced by illuminating the cultures. Further, that autoclaved tryptophan very nearly reproduces in dark-grown cultures the enhancement of growth achieved by illumination. In this case, however, indolylacetic acid does not effectively substitute for tryptophan; the wheat roots are apparently much less able to metabolize indolylacetic acid to the required active compound.

Now it is known that, under certain conditions, the growth of excised roots of maize[33], *Lycopersicum pimpinellifolium*[34] and tomato[35] is also enhanced by low-intensity illumination, and work on the action spectrum of this light effect with both tomato and wheat indicates a peak of activity in the red. This work prompts me to ask whether light is an essential requirement for the growth of all roots in culture; in which case the roots of most species so far successfully cultured presumably receive sufficient illumination during the manipulations involved in subculture to permit active growth during the subsequent dark incubation period. If this is so, then it can be suggested as one possibility that the growth of roots normally depends on the translocation to them of some specific hormone synthesized in the shoot; the photochemical synthesis of this hormone can, however, also be achieved in root cells exposed to light. In the light-responsive excised wheat root we may have a system in which it will be possible to characterize and study the biosynthesis of the hypothetical 'rhizocaline' (root hormone supplied by the shoot) first so called by Bouillene and Went[36] but afterwards invoked by a number of workers.

This review, despite its incompleteness, may nevertheless have shown that our knowledge of the hormonal control of root growth has reached a point which could lead to very fruitful further research. This will, however, require the isolation and identification from root extracts of active hormones which, on present evidence, can be expected to be present in very low concentrations; the isolation of the enzymes concerned in the synthesis and metabolism of these growth hormones and the intensification of physiological studies. If such studies are to be pursued with sufficient intensity to have a successful outcome it will require a considerable re-orientation of the research resources at present devoted to the study of what may be broadly called the field of plant hormone research, a field in which extensive practical applications are based upon a most slender body of fundamental knowledge.

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OBITUARIES

Mr. A. L. Hetherington, C.B.E.

Arthur Lonsdale Hetherington, who died on August 21 at the age of seventy-eight, was educated at Highgate School and Trinity College, Cambridge. On leaving Cambridge, he taught for two years at his old school and then served for two years in Burma in the Indian Educational Service. He returned to Britain in 1908 as a junior examiner in the Board of Education. He acted as secretary of the Board's Advisory Committee on University Grants and as assistant secretary to the committee which framed the scheme of reconstruction for the University of London. During 1914–19 he was secretary of the Carnegie United Kingdom Trust.

In 1919, Hetherington was appointed to the Department of Scientific and Industrial Research, but was immediately loaned to the Treasury to act as secretary of the newly formed University Grants Committee. He was promoted assistant secretary in

1922 and until his retirement in 1943 was in charge of the grants to postgraduate students for training in research, to individual research workers and the grants to the co-operative research associations. He thus assumed responsibility, without any industrial background, for the research association movement in Britain at the most critical and difficult period of its history.

The novelty of this scheme for Government assistance to research in industry was wearing off, and the post-war boom years were coming to an end. Industry was generally indifferent to science and often suspicious of the co-operative aspect of the associations. Money was tight both in industry and Government, and the associations were compelled to function with very limited resources. Most of them were doing useful work, as evidenced by the book Hetherington wrote with Sir Frank Heath on their early history; but the results achieved were seldom of a kind to make a ready appeal to industrialists imbued with a profound faith in traditional methods. It was largely due to Hetherington's skill as an administrator and to his personality that the movement survived these lean years to play a notable part in the Second World War. His work demanded much tact and patience—virtues which did not come to him naturally. It was a great satisfaction to him to see the associations expand after the War to occupy the important position in British industry they hold to-day.

Hetherington's early training fitted him admirably to deal with the scientific grants to individuals; but even here he had his difficulties, as during the 30's more than one influential, but unenlightened, Civil Servant made no secret of his opinion that many of the grants, especially those for training research students, were little more than unemployment doles.

Hetherington's official work was lightened by two hobbies. The first was his keen interest, as collector and expert, in Chinese porcelain. In this field his important book, written with R. L. Hobson, on "Early Ceramic Wares of China" brought him an international reputation. He also took a leading part in the formation of the Oriental Ceramic Society. His second interest was in turning on the lathe. Here recognition of his skill led to his becoming a Master of the Turners' Company, the honorary freedom of which was bestowed on him in 1932. He took special interest in the turning properties of many little-known Empire timbers.

Finally, Hetherington had quite a talent for versification, and frequently amused his colleagues with mildly satirical verses on matters of topical interest to the Department of Scientific and Industrial Research.

O. F. Brown

Dr. W. D. Douglas

The death on August 5 of Dr. W. D. Douglas removes another of the now thinning ranks of first-generation aircraft scientists. He was born in Ireland on August 2, 1887, and educated as an electrical engineer at Trinity College, Dublin. For some years he served as a hospital X-ray operator, and then joined the staff of the Royal Aircraft Establishment and specialized in the properties of materials, especially timber and adhesives, which were the common materials of aircraft structures. He became a leading authority on their properties.

In the 1930's, he was in charge of the mechanical testing of aircraft structures and components and materials. Under his guidance the science of testing large structures grew from small beginnings to something like its present degree of sophistication. His advice on the design and strengthening of structures was eagerly sought by aircraft designers.

Douglas's knowledge of the mode and cause of failure of materials was invaluable to the Air Ministry's Accident Investigation Department, and it became usual for the pieces of crashed aircraft to be sent to him. After painstaking re-assembly and examination of scratches, dents and fractures, he was often able to decide the sequence of catastrophe and the cause of failure. The techniques he developed form the basis of present-day aircraft accident investigation. Re-organization during the Second World War put Douglas in charge of the Materials Department at the Royal Aircraft Establishment, the three divisions of which covered chemistry, metallurgy and non-metallic materials.

On retiring from the Civil Service in 1946, Douglas joined the furniture firm of Harris Lebus as its first head of research. In this unique position he spent nine fruitful years, asking questions, seeking answers and giving sound advice. He represented the firm on technical committees, and when the Furniture Development Council was started in 1949, Douglas joined its research committee and was most helpful in formulating programmes and in guiding the Council's research officers. He played a major part in the development of the performance tests for furniture, which are now incorporated in British Standard Specifications. Furniture satisfying the standards is marketed under the 'Kite' mark.

Douglas retired from Harris Lebus in 1955, but continued to serve most effectively on the Furniture Development Council.

In personal characteristics he was a modest man, and the most helpful and sincere of friends to those in need; but he could also be a worthy adversary. A devoted husband and father, he leaves a widow and son.

C. Gurney

Dr. Peter L. Goldacre

Dr. Peter L. Goldacre, a senior research officer in the Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organization, Canberra, Australia, died after a brief illness on April 16, at the age of thirty-four. Paradoxically, at the time of his death from cancer, he was engaged in incisive and rewarding investigations into the nature of the chemical factors controlling cell division in plants.

Goldacre was educated at Sydney Boys' High School and the University of Sydney. He joined the Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organization, in 1947, as one of the first two plant biochemists to be appointed to this agency. His early work, reflecting his biochemical training, was largely enzymological. He was the first to characterize the 'indoleacetic acid oxidase' complex as being composed in part of a peroxidase. Later, at the California Institute of Technology, where he obtained his Ph.D. degree in 1952, he was instrumental in discovering the activating effect of substituted monophenols on this enzyme complex. During these investigations, he also discovered that 2,4-dichlorophenol is a specific inhibitor

for the enzyme catalase, and thus permits the demonstration of the related enzyme peroxidase in such tissues as the liver, where its presence is normally masked by the preponderance of catalase.

By the time he returned to Australia, Goldacre had become keenly aware that many important questions in biology were not ready for investigation by classical biochemical techniques. He made the important and difficult decision to shift his research emphasis to the mechanism underlying differentiation of plant cells. His experiments on the auxin-induced initiation of lateral roots in cultured excised flax roots convinced him of the existence of a cell division inducer (kinin) elaborated by the inhibited, pre-existing lateral root primordia. His search for rich sources of kinin-like materials in tissues undergoing cell division led to the discovery of 'Kineapple' (*Nature*, **184**, 555; 1959), a yet uncharacterized, highly active inducer of cell division from young apple fruitlets. In collaboration with several colleagues in Canberra, he was attempting the isolation of this material from three-quarters of a ton of apple fruitlets at the time of his death.

Peter Goldacre's outstanding characteristics were his honesty and easy-going informality, together with his impatience with pretension and sham. He loved camping and the outdoor life; he had a powerful physique and excelled at swimming and other sports. Six months before his fatal illness, he had attended the Fourth International Congress on Plant Growth Regulation at Yonkers, N.Y., and appeared in vigorous good health. The sudden knowledge of his impending death did not materially alter his essentially humorous and compassionate outlook on life.

He leaves his wife and three young children, the youngest born only a few months before his death. To those privileged few who knew him well, he will be remembered as one of Nature's noblemen. The tragedy of his early death lies in the fact that he had so much to give to many who had not yet come to know him.

Arthur W. Galston

NEWS and VIEWS

Education in Industry

The British Association for Commercial and Industrial Education has issued reports on two of its recent conferences. The first, "Education for Survival", contains the papers presented at the proceedings of the East Midland Group and is noteworthy for a stimulating account of technological education in Britain by Dr. B. V. Bowden, principal of the Manchester College of Science and Technology. The list of members attending indicates that speakers on subjects like the training of non-apprentices and the attitude of trade unions to apprenticeships were preaching mainly to the converted. The Association would perform a valuable service to industry if it could persuade recalcitrant managing directors of the need for training apprentices and others. The second conference dealt with "The Implications of the Crowther Report", and the speakers, who included the Minister of Education, underlined its main findings and recommendations. The value of what might have been a useful report is diminished by its appearance some five months after the Conference. Information about both reports may be obtained from the Director, British Association for Commercial and Industrial Education, 26*a* Buckingham Palace Road, London, S.W.1.

British Rubber Producers' Research Association

The current report of the British Rubber Producers' Research Association gives an account of the progress made during 1959, both in the fundamental study of the structure and properties of natural rubber and in the application of scientific knowledge acquired in recent years to the problems of the industry (Twenty-second Annual Report. Pp. 53. 1960). On the fundamental side, work on the biosynthesis of rubber in the tree has been made possible by the installation of a tropical greenhouse at Welwyn Garden City. The attack on this elusive problem includes the study of the structure of the latex vessels by the electron microscope, and the chemical examination of cell constituents. The chemical processes of vulcanization, that is, of the formation of a network of long-chain molecules, and the converse processes of ageing or network breakdown continue to be the subject of intensive study; progress in this field, though necessarily slow, is continuous. Of particular interest is the elucidation of the mechanism of surface cracking by ozone and the role of anti-ozonants. It is well known that this type of degradation is dependent on the state of stressing of the rubber, and it has now been shown that the effect of the anti-ozonant is to raise the critical stress for crack growth, while leaving the rate of growth above this critical stress unchanged. The somewhat related problem of fatigue failure has been shown to be associated with the growth of flaws having dimensions of the order of 0·01 mm. The report gives examples of the application of the knowledge obtained from these fundamental investigations to the production of rubbers and rubber compounds having more desirable physical and chemical properties. This work is designed to ensure that natural rubber is not left behind in the competition with the synthetic materials now available. The report is available from the Association, 19 Fenchurch Street, London, E.C.3.

Comparative Efficiency of Indexing Systems

A report by Mr. C. W. Cleverdon on the first stage of an investigation into the comparative efficiency of indexing systems, an investigation supported by a grant to the Association of Special Libraries and Information Bureaux by the National Science Foundation, has now been issued by the College of Aeronautics, Cranfield, on behalf of Aslib (Pp. v+166. Cranfield: The College of Aeronautics, 1960). The grant of £10,000 was made in July 1957 for a study of the comparative efficiency of the Universal Decimal Classification, the alphabetical subject catalogue, a faceted classification system, and the Unitern system of co-ordinate indexing, which in this first stage involved the indexing of 18,000 research reports and periodicals in the general field of aeronautical engineering, about half of which dealt with high-speed aerodynamics. The present

report is essentially a factual account of this first stage and any firm conclusions must await the result of using a piece of test equipment in a test programme for which the National Science Foundation has awarded a further grant. Meanwhile besides describing the general organization of the programme and giving a detailed analysis of the problems encountered, the report includes various statistical details and an account of some supplementary indexing undertaken by other organizations and individuals in co-operation. When the test programme is completed it should be possible to analyse the results so as to ascertain in detail the reasons for success or failure in locating information and eventually to state with reasonable certainty exactly what requirements an information retrieval system must meet and propose methods for the design of such a system.

Co-ordination of Research

A VALUABLE means of co-ordinating research in engineering over an 'academic' region is being carried out by the Academic Board for Higher Technology of the Northern Advisory Council for Further Education (Thirteenth Annual Report, 1959/1960. Pp. 32. Newcastle upon Tyne: Northern Advisory Council for Further Education, 1960). The Board has accepted proposals put forward by Prof. W. Fisher Cassie, of the University of Durham, in which he recommended that the institutions for higher technology in the region should be asked to send to the Board their proposals for research projects. In this way, "the Board would be informed of the whole pattern of research and would be able to advise on the use of expensive apparatus and on the possibility of a number of institutions sharing in a research of some magnitude". The Board also accepted recommendations put forward by Prof. A. F. Burstall that more provision should be made for training in engineering design. As a first step a sub-committee was appointed to report on the organization of short courses for designers. Industry will warmly welcome this initiative.

Magnetic Data for the International Geophysical Year

THE 1957 and 1958 volumes of the Observatories' Year Book covering the International Geophysical Year have recently been published (Air Ministry, Meteorological Office. The Observatories' Year Book. 1957. Pp. vi+120. (M.O. 680.) 50*s*. net. 1958. Pp. vi+128. (M.O. 681.) 55*s*. net. London: H.M. Stationery Office, 1960). They contain the autographic records and eye observations of terrestrial magnetism at Lerwick and Eskdalemuir observatories and of atmospheric electricity at Lerwick and Kew. They also contain the log of the auroral watch at Lerwick and a general auroral table for the British Isles. These two volumes have been published out of turn, the otherwise latest volume published being that for 1950, in order to make the fundamental magnetic observations made at the Scottish observatories during the International Geophysical Year available to geophysicists as early as possible. The volumes for 1951–56 are expected to be published during the next twelve months.

The Sarawak Museum Journal

THE *Sarawak Museum Journal*, under the enthusiastic editorship of Mr. Tom Harrisson, Government ethnologist and curator of the Museum, is always of interest to many outside Borneo. The current issue (9, No. 13–14 (New Series), July–December 1959) includes papers on ethnology, history, prehistory, Welfare States, natural history and some book reviews. Notes on "An All-round Museum in a small Country" are as applicable elsewhere as they are in Borneo. Other articles include some carbon-14 datings from Niah caves, an account of the Piltdown forgery from the point of view of orang-utang teeth from Borneo or Sumatra, and notes on cock-fighting, the Dayah national game.

Education in the Primary School

To help teachers in primary schools, the National Union of Teachers is issuing a new series called "Curriculum Studies". The first, "Mathematics in the Primary School", has been prepared by R. A. Swallow, who has specialized in the teaching of mathematics to primary school children over many years. The booklet is clearly written, full of problems which can be presented to children at differing ages, and based on his own experience of children. The second, "Science in the Primary School", explains how science in the primary school should be developed and describes means of doing this. The author, Cedric Griggs, should now follow the sequence of Mr. Swallow's booklet and present a programme of science teaching to develop coincidentally with young children's emerging interests. Both booklets can be obtained from the Schoolmaster Publishing Co., Hamilton House, Hastings Street, London, W.C.1, the former price 2*s*. 6*d*. and the latter 2*s*.

New Technique in Insulation Testing

THE new 'Megger' insulation tester, series 3, mark 3, manufactured by Evershed and Vignoles, Ltd., incorporates some advanced features made possible by new manufacturing techniques and materials. The instrument consists of a hand-driven generator and a direct-reading ohmmeter. The moving element of the ohmmeter consists of two coils, the control and deflecting coils, arranged so as to oppose each other. There is no control spring and the deflexion of the movement depends on the ratio of the currents in the two coils. The turning effort of the new instrument is considerably less than that required for earlier models. A clutch-less nylon gear-train transmits the handle torque to the generator and provides remarkably smooth and consistent operation. In addition to the insulation testing range, the instrument operates over a continuity range 0–100 ohms with a scale of shape permitting readings as low as 0·1 ohm. The continuity testing voltage is obtained from a new miniature generator so that no battery is required and thus constant readiness for use is ensured.

Evaluation and Presentation of Spectro-Analytical Results

IN the pocket booklet entitled "Evaluation and Presentation of Spectro-Analytical Results" the author, A. B. Calder, directs attention to the various types of error which arise in chemical and instrumental analysis (London: Hilger and Watts, Ltd., 1959. 5*s*. net). The errors are classified into two groups, determinate or constant errors, and indeterminate or accidental errors. Their mathematical treatment is dealt with in some detail and then finally the application of the treatment to various spectro-analytical problems is discussed. Throughout, references are given to fuller treatments,

but sufficient warning and guidance are given to indicate to the reader the statistical nature of certain problems encountered in analytical spectroscopy. The importance of indicating how the errors are distributed in multi-stage analytical techniques and the saving in operational time which can often be attained, without loss of accuracy, by the correct interpretation of the analytical data, are emphasized.

Hydrogen in Jupiter

Although molecular hydrogen has long been regarded as a constituent of the atmosphere of Jupiter, direct spectroscopic evidence has been lacking. Jupiter has a known mass and size, and the resulting calculated mean density is surprisingly low. When this was considered in relation to the central condensation of the planet as determined from the motions of its satellites, it became clear that much of the planet must be made up of a light substance. The surface gravity on Jupiter is sufficient to retain light gaseous elements, and it seemed likely that hydrogen might be an important constituent at least of the outer layers of Jupiter, and perhaps also of the interior. The spectroscopic detection of molecular hydrogen has proved to be extremely difficult, and it is only possible if a very large quantity of hydrogen is present. The detection rests on a few extremely faint lines of the quadrupole rotation–vibration spectrum of the molecule. It has now been accomplished in the spectrum of Jupiter by C. C. Kiess, C. H. Corliss and Harriet K. Kiess (*Astrophys. J.*, **132**, 221; 1960), who found four of the quadrupole lines. In their paper the authors also direct attention to an unexpected excess continuous absorption, which is so far unidentified, but which closely resembles a known nitrogen tetroxide absorption. Objections to this identification can be raised on the grounds that the hydrogen would act on the tetroxide as a reducing agent, but no other identification has yet been suggested.

Banana Fruit Development

N. W. Simmonds has reported on experiments on the development of banana fruits and seed fertility, various synthetic auxins and auxin-antagonists being applied to young fruits in lanolin emulsions (*Ann. Bot.*, N.S., **24**, 212; 1960). Auxins stimulated the formation of edible pulp in both seedy and parthenocarpic bananas and induced varying degrees of seed fertility. Of the auxin antagonists tried, coumarin stimulated parthenocarpy and induced seed sterility, three others were not or only slightly active, and thiourea markedly inhibited parthenocarpy. Study of the relation between size of mature fruit and seed content indicates that developing seeds stimulate the growth of pulp, and that the amount of stimulation is roughly proportional to the level of parthenocarpy. Fruit development in the banana seems to fit in with Luckwill's general scheme of dual hormonal stimulus.

Pharmacology of Psychotropic Drugs

The number of chemical compounds with an effect on the higher centres of the central nervous system has greatly increased in recent years; and the remarkable properties of some of them have aroused much interest in their modes of action, which are now better understood because of improved knowledge of the physiology of the central nervous system. A paper by the Director of Research, Dumex, Ltd., Copenhagen, examines systematically the effects of some of the better known of these psychotropic drugs on the various levels of the central nervous system; their antagonistic and synergistic effects; and the relationship between these effects and the effects on other functional systems and on behaviour. Although the picture revealed is complex and there are many gaps, psychotropic drugs fall into certain classes according to the effects they have, and a system of classification could be developed on this basis. ("The Comparative Pharmacology of Some Psychotropic Drugs". E. Jacobson. Offprint from *Bull. World Health Org.*, **21**; 1960. 5*s.*, 1 dollar or Sw. Fr. 3.)

Nomenclature of Human Chromosomes

The discovery that some errors of sexual development and other congenital defects in man were associated with karyotypic abnormalities has aroused considerable interest, and research in this field is growing rapidly. Already four different states of numerical unbalance of the sex chromosomes have been identified, as well as three primary autosomal trisomic conditions, several examples of karyotypic mosaicism, and other cases attributable to reciprocal translocation and to deficiency. Various systems of numbering of the chromosomes have been used by different research groups, making the comparison of their results difficult. A meeting with the object of trying to devise an agreed nomenclature was therefore arranged by Dr. T. T. Puck, of the University of Colorado, and held in Denver during April 9–11. All those who had contributed to studies of human somatic chromosomes up to the end of 1959 were invited to take part and nearly all were able to attend. Prof. D. G. Catcheside (who acted as chairman), Prof. H. J. Muller and Prof. C. Stern were also present as councillors. A system was agreed on and a statement embodying it and other information relating to the identification of human mitotic chromosomes has been published (*Lancet*, i, 1063; 1960). The statement was signed by all the members, who included five from the United States, three from Britain, three from Sweden, one from France and one from Japan, in addition to those already mentioned. It is hoped that the proposed standard system will be generally adopted, and that any who may prefer to use another scheme will refer to the proposed system and show clearly in what way their own system differs.

Bibliography of Indology

The National Library, Calcutta, has published the first volume of "A Bibliography of Indology" (Vol. 1: Indian Anthropology. Compiled by J. M. Kanitkar. Edited, revised and enlarged by D. L. Banerjee and A. K. Ohdedar. Pp. xi+290. (Calcutta: National Library, 1960.) Rs. 5.00. 8*s.*). It is in fact a catalogue of more than 2,000 publications on the archæology and anthropology of the sub-continent, with frequent notes and extracts from reviews describing the contents of the works. Added are author and subject indexes which enable the reader to use conveniently the references. The result is a very useful bibliography, if not entirely complete, particularly on the prehistoric side.

University News: Belfast

A contract has been signed between the Queen's University of Belfast and the United States Navy European Research Contracts Office. Under this contract, in the first year, the University will be supplied with a *Deuce* electronic digital computer and

with money for additional research staff. In return, Prof. D. R. Bates, Dr. A. Dalgarno, and several other members of the Applied Mathematics Department will undertake theoretical investigations on some problems of importance to the Advanced Research Projects Agency (on behalf of which the U.S. Navy Office is acting). In anticipation of the signing of the contract the erection of a building for the computer has been authorized and a new lectureship in digital computing has been established. A digital computing unit has been set up with the Applied Mathematics Department, and Dr. Dalgarno has been appointed director of this unit.

Birmingham

THE University has accepted the offers of Harry H. Payne, Ltd., and of the Harry Payne Trust to make further payments of £1,250 per annum and £1,000 per annum, respectively, to the Harry Payne Fund for a period of seven years from 1961. The Harry Payne Fund was established in the University in 1954 with gifts of £1,000 per annum each from Harry H. Payne, Ltd., and the Harry Payne Trust for seven years. The Fund is used to support a lectureship in employer/employee relations. The lectureship is held by Dr. N. S. Ross, senior lecturer in employer/employee relations.

The following appointments to lectureships have been made: Dr. G. M. Bennison (geology); Dr. T. S. M. MacLean (electrical engineering); Dr. D. A. Wilkins (botany).

London

DR. P. E. POLANI has been appointed to the Prince Philip chair of pædiatric research tenable at Guy's Hospital Medical School. The title of reader in hæmatology in the University of London has been conferred on Dr. Sidney Shaw in respect of his post at Charing Cross Hospital Medical School, and that of reader in bacteriology on Dr. H. I. Winner in respect of his post at the same School.

Massachusetts Institute of Technology: Department of Nutrition, Food Science and Technology

THE Food Technology Department of the Massachusetts Institute of Technology is to be enlarged and renamed the Department of Nutrition, Food Science and Technology. Dr. N. S. Scrimshaw, director of the Institute of Nutrition of Central America and Panama and regional adviser on nutrition of the Pan American Health Organization (the Regional Office for the Americas of the World Health Organization), has been appointed head of this Department.

Dr. Scrimshaw, who is at present an adjunct professor of public health nutrition in Columbia University and a visiting lecturer in the Harvard School of Public Health, will assume his new duties on August 1, 1961. He will continue his association with the Institute of Nutrition of Central America and Panama as a technical director.

The Night Sky in November

FULL moon occurs on Nov. 3d. 11h. 58m. U.T., and new moon on Nov. 18d. 23h. 47m. The following conjunctions with the Moon take place: Nov. 5d. 18h., Aldebaran 0·4° S.; Nov. 9d. 00h., Mars 6° N.; Nov. 21d. 15h., Jupiter 5° S.; Nov. 21d. 19h., Venus 7° S.; Nov. 22d. 08h., Saturn 4° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Jupiter on Nov. 19d. 02h., and with Saturn on Nov. 28d. 07h., on both occasions Venus being 2° S. Mercury is too close to the Sun at the beginning of the month for normal observation, but it becomes visible as a morning star later in the month; it will be visible low in the south-east before sunrise, rising about two hours before the Sun on November 23. There is a transit of Mercury on November 7, partly visible at Greenwich. At ingress, exterior contact takes place at 14h. 34·4m., and interior contact at 14h. 36·4m. Only the earlier part of the transit will be visible in Great Britain; sunset occurs at 16h. 20m. at Greenwich and Mercury leaves the solar disk at 19h. 13m. The last November transit of Mercury was in 1953, and the next will be in 1973. Telescopic aid will be necessary to observe the transit. Venus is an evening star, setting at 17h. 50m., 18h. 00m. and 18h. 30m. on November 1, 15 and 30, respectively. During this time its distance from the Earth decreases from 119 to 102 million miles, the visible portion of the apparent disk is from 0·823 to 0·744, and its stellar magnitude from −3·4 to −3·6. Venus is visible in the south-west after sunset. Mars rises at 20h. 05m., 19h. 20m. and 18h. 10m. at the beginning, middle and end of the month, respectively. It is in Gemini, reaching stationary point on November 21 and then beginning to move westward among the stars. Its stellar magnitude is −0·5 and its distance from the Earth on November 15 is 69 million miles. Mars is now a very prominent object and can be seen almost all night. Jupiter is low in the south-west at sunset, and sets about two hours after the Sun. Conditions are unfavourable for observation. Saturn is in Sagittarius, east of Jupiter, and sets about three hours after the Sun. Conditions are unfavourable for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 6d. 5h. 05·6m., 318 B. Tau. (*R*); Nov. 15d. 4h. 02·0m., η Vir. (*R*). *R* refers to reappearance. The Taurid meteors are active near the beginning of the month, but conditions are unfavourable. The Bielids are active about November 14, with a radiant near R.A. 1h. 32m., Dec. +43°. This shower has recently been recovered photographically, and conditions are favourable, so that there is hope that it may again be seen visually. The Leonids are active on November 15–17, conditions being favourable; the radiant is near R.A. 10h. 08m., Dec. +22°.

Announcements

PROF. REBECCA C. LANCEFIELD of the Rockefeller Institute has been awarded the third T. Duckett Jones Memorial Award of the Helen Hay Whitney Foundation. The award, amounting to 6,500 dollars, was conferred for "her long and continuing studies which are so largely responsible for the present knowledge of the biology of hæmolytic streptococci".

PROF. JOHN MCMICHAEL, professor of medicine in the University of London at the Postgraduate Medical School, Hammersmith, has been appointed a trustee of the Wellcome Trust in place of Sir Henry Dale, who retired from the Trusteeship in July 1960, and was succeeded as chairman by the Rt. Hon. the Lord Piercy. Sir Henry Dale will continue to serve the Trust in an advisory capacity as scientific consultant.

ERRATUM. In the article entitled "Dwarf Mistletoes" in *Nature* of October 8, p. 112, the author of the paper surveyed is incorrectly given as "J. Knight"; the paper is in fact by Dr. Job Kuijt.

SCIENTISTS IN THE PUBLIC SERVICE

SPECIAL PROMOTIONS

SPECIAL posts have been created in the Civil Service as in previous years under provisions included in the White Paper on the Scientific Civil Service (Cmd. 6679, 1945) to provide for the promotion of individual research workers of exceptional merit. The promotions were effective from July 1, and include the following.

Deputy Chief Scientific Officer

MR. R. BENJAMIN joined the Admiralty Signal Establishment in 1944. During the following years his many contributions to radio and radar research and development were notable for their remarkable insight and brilliant execution. Promotion was rapid and he became a special merit senior principal scientific officer, in 1955, at the age of 32. By that time he was already established as an international authority on the theory, design and use of circuits in the video and pulse fields. His practical application of this knowledge to the processing and display of information, together with a major contribution to the concept of the accompanying radar, have borne fruit in the latest naval 3-D air-defence system which has been demonstrated with such conspicuous success. In addition to a continuing concern with advanced radar systems since 1955, Mr. Benjamin has been exploring new techniques of automation and computation to be applied to data processing and weapon control in virtually the whole field of naval warfare.

MR. L. G. CARPENTER, head of the Metallurgy Department, Royal Aircraft Establishment, joined the Establishment as head of the Research Section of the Instrument Department in 1937, and in 1940 was responsible for development of the first gyro-stabilized bomb site to go into Royal Air Force use. In 1940 he was transferred to the charge of the Research Section of Armaments Department concerned with bomb fuzes, bomb ballistics and the air-to-ground rocket weapon. In 1944, he was appointed head of the General Armaments Division and in 1946 head of the High-Temperature Materials Group in the Metallurgy Department. This group was the first (1947) in the United Kingdom to prepare titanium in a pure state and determine its properties. He has guided research on metal fatigue, properties of materials at high temperatures, the use of graphite for the hot parts of rockets and as a fuel container for nuclear reactors and the study of gas/solid reactions. His recent personal work has been concerned with the absorption of oxygen and nitrogen by hot titanium and the oxidation of gold. He is at present engaged on the reaction of rarified beams of nitrogen with solid surfaces and the required vacuum techniques.

DR. L. ESSEN, of the National Physical Laboratory, is internationally well known for his pioneer work on precise frequency standards for accurate measurements of frequency and time-interval. His first major contribution, the Essen quartz-ring oscillator, proved greatly superior to any previous frequency standard and to any mechanical clock for precise time-keeping in observatories and standard laboratories. More recently he was responsible for introducing the cæsium atomic beam resonator as a natural standard with a frequency stability and reproducibility of 1 part in 10^{10}. With his co-workers in Britain and in the United States he has used it to calibrate quartz clocks and monitor standard frequency transmissions, thereby establishing invaluable data on the performance of quartz clocks and different cæsium standards, the propagation of radio signals, variations of the Earth's rate of rotation and the value of the second of (ephemeris) time in terms of the cæsium standard. His determination in 1947 and 1950 of the velocity of propagation of microwave radiation revealed the fact, since confirmed by other observers in the microwave and optical regions, that the previously accepted value of the velocity of light was too low by 1 part in 20,000. His work as an experimental physicist has been nationally and internationally recognized by several awards and he was recently elected a Fellow of the Royal Society.

DR. C. TIMMS joined the scientific staff of the Metrology Division at the National Physical Laboratory in 1936. There he worked with Dr. G. A. Tomlinson, whose work on the standards of accuracy of gear-cutting machinery he has since continued and developed. This work played a large part in the marked improvement effected during the Second World War in the accuracy and reliability of naval and marine reduction gear drives. He is now a national authority on all aspects of gear metrology, and recently was invited to give the annual Viscount Nuffield Lecture to the Institution of Production Engineers on this subject. He has also carried out fundamental work in the measurement and assessment of surface finish, and has been largely responsible for the development of the branch of precision engineering measurement referred to as dynamic metrology. Dr. Timms became head of the Mechanisms, Metrology and Noise Control Division of the National Engineering Laboratory in 1950, and is now applying his knowledge and experience to improvement of the performance of machine tools, particularly in the application of precision circular diffraction gratings to measurement and correction of errors.

Senior Principal Scientific Officer

DR. R. N. COX of the Armament Research and Development Establishment has a wide background of research in experimental and theoretical fluid dynamics and is an expert in the field of hypersonics research.

Dr. R. G. Hopkinson is in charge of that part of the Building Research Station research programme which deals with the lighting of buildings; his particular research interest is the psycho-physics of illumination in relation to design for daylighting and artificial lighting. Good lighting has an important influence on building design, and Dr. Hopkinson's work has contributed greatly to the definition of the quality of lighting on a metrical basis.

Mr. D. G. King-Hele of the Guided Weapon Department, Royal Aircraft Establishment, is a versatile mathematician, whose work on satellite orbits is now internationally known.

Dr. D. McLean of the Metallurgy Division, National Physical Laboratory, is engaged on the investigation of dislocation structure in iron and iron alloys using the thin-film technique in the electron microscope.

Mr. E. C. Maskell of the Aerodynamics Department, Royal Aircraft Establishment, is an outstanding aerodynamics worker, particularly in the field of slender wings and jet flaps.

Mr. W. R. Piggott joined the Department of Scientific and Industrial Research in September 1939. After an early association with Sir Edward Appleton in certain special radio projects during the War, he joined the staff of the Radio Research Station and began his studies of the ionosphere for which he is so well known. Mr. Piggott has made an intensive investigation of the absorption of radio waves by the ionosphere, and pursued this and related topics in great detail during the International Geophysical Year: in the latter respect he has made valuable analyses of the observations made at Halley Bay by members of the Royal Society Expedition. His outstanding contribution to knowledge of the upper atmosphere is demonstrated by the numerous papers he has published, including a special report issued by the Department concerning the design of aerials to take the best advantage of the ionosphere for long-distance transmission.

Mr. L. P. Smith has been head of that section of the Meteorological Office which specializes in meeting the requirements of agriculture and horticulture since 1949. His success in this form of operational research has been noteworthy, and he has made many practical and easily assimilated contributions to good husbandry. His studies have included relationships between weather conditions and the spread of blights and diseases of plants and animals.

Dr. N. Uri joined the Research Establishment and Experimental Factory, Aberdeen, of the Ministry of Agriculture, Fisheries and Food in 1954 as a Senior Government Research Fellow, and was established as a principal scientific officer in 1956. The applied objectives of his work have been the prevention of deterioration by oxidative rancidity in food, particularly foods with low water content produced by freeze-drying. He has developed a theory on the effect of the polarity of the environment on trace metal catalysis in relation to the onset and speed of oxidative rancidity in fats. He has devised a new method of testing the stability of fats based on metal catalysis. He has also investigated the anti-oxidant properties of naturally occurring substances such as quercetin and the synthesis of new anti-oxidants.

Mr. W. Watt of the Metallurgy Department, Royal Aircraft Establishment, is a research worker in the field of high-temperature materials, especially ceramics and interstitial graphite.

Similar promotions have been made by:

(1) Agricultural Research Council: Senior Principal Scientific Officer

Dr. R. A. Beatty of the Unit of Animal Genetics, Edinburgh, is engaged on work in the common ground between genetics, embryology and reproductive physiology.

Dr. R. M. C. Dawson of the Biochemistry Department, Institute of Animal Physiology, Babraham, Cambridge, works on phospholipids and their metabolism.

Dr. R. F. Glascock is head of the Department of Radiobiochemistry at the National Institute for Research in Dairying, Reading, and has specialized in the application of the isotopic tracer technique to biochemical problems.

Dr. C. G. Johnson is a member of the Entomology Department of the Rothamsted Experimental Station, Harpenden, Herts. He is best known for his work on the aerial dispersal of insects.

(2) U.K. Atomic Energy Authority: Senior Principal Scientific Officer

Dr. T. E. Cranshaw has worked at Harwell for ten years. During the first eight years he worked on extensive air showers produced by high-energy cosmic rays. In 1954 he designed and built the apparatus at Culham Airfield which was the first to use the principle of covering a large area by a lattice of identical units, and with which many properties of air showers were investigated. In 1959, Dr. Cranshaw performed an experiment to measure a possible difference between the charges of the proton and the electron, which was shown to be smaller than one part in 10^{20}. More recently he has worked on the Mössbauer effect and its application to relativistic frequency shifts.

Mr. R. D. Lowde, during his thirteen years at the Atomic Energy Research Establishment, Harwell, has been in particular an exponent of neutron inelastic scattering techniques as a means of studying the solid state. He was the first to demonstrate the crystallographic phenomena that are the basis of the modern neutron-diffraction methods of investigating lattice vibrations and the dynamics of spin systems in magnetic substances. In recent years he has carried through a series of investigations in the theory and practice of these methods; the principal results have been the finding of effects due to 'spin waves' in metallic iron and due to magnetic 'critical phenomena' near the Curie temperature of the same material. In addition to his work in neutronics and magnetism, he has published researches in electronics and crystallography, and many papers on scientific instruments and methods.

(3) Nature Conservancy: Senior Principal Scientific Officer

Mr. M. V. Brian is officer in charge of the Furzebrook Research Station of the Nature Conservancy. He is well known for his contributions on social insect populations.

ASTRONOMY IN CANADA

THE April and June issues of the *Journal of the Royal Astronomical Society of Canada* (**54**, 79, 126; 1960) contain abstracts of reports read to the National Committee for Canada of the International Astronomical Union at a meeting held in Ottawa on November 27, 1959. These reports give a comprehensive view of research programmes at present being undertaken by Canadian institutions, and testify to the virility of Canadian astronomy.

The Dominion Observatory at Ottawa has made important contributions to positional astronomy, and this work is continuing. Two programmes are international in scope, being shared by meridian circle observers at fifteen observatories. These are the observation of 1503 *FK*3 supplementary stars, and a programme of 3754 *AGK*3 reference stars required for the photographic survey of improved positions and proper motions. Much work is done in connexion with the Time Service, including a co-operative effort to determine the relation between Universal Time and Ephemeris Time by direct lunar photography using a Markowitz Moon camera. Work at Ottawa is by no means confined to positional astronomy, and investigations are in progress on meteor astronomy and meteorites, solar physics and radio astronomy. An intensive search of air photographs of Canada has revealed a number of circular features, some of which may be the remains of meteorite craters. These are being studied by geophysical techniques, and in two cases rock cores have been drilled. It is considered highly probable that the craters at Holleford, Ontario, and Deep Bay, Saskatchewan, are due to meteoritic impact. An 84-ft. radio telescope has been erected near Penticton, B.C., and installation of equipment is in progress. A programme on 21-cm. hydrogen radiation will soon be undertaken.

At Queen's University a modest programme of astronomical research has been started. Measurements on the scintillation of radio sources have produced results relating to the ionosphere, to rapid variations of the geomagnetic field, and to radio bursts from the Sun. Much radio equipment is in the course of construction, with the view of counting cosmic radio sources.

The David Dunlap Observatory of the University of Toronto is the leading Canadian institution with a large teaching schedule in astronomy. Recently there has been a marked increase in student enrolment in astronomy courses, and there are at present five students taking the full graduating option in astronomy, and six graduate students. Recently accepted theses have included calculations on the interior structure of sub-dwarfs, and photometric and spectrophotometric studies of visual binaries and the determination of their ages. Some work on stellar radial velocities is still in progress, although no new large programmes in this field have been laid out in recent years. The long-established work on globular clusters is continuing; about 4,500 photographs have been accumulated. Many cluster variable stars have been found and periods determined. A supplementary bibliography on individual clusters is being prepared and will shortly be published. Spectroscopic studies of hot carbon stars have been made, and it has been shown that the photo-ionization of neutral carbon is probably responsible for the opacity in the atmosphere of *R* Coronae Borealis. Investigations of galaxies have led to the identification of a large near-by cluster of galaxies in Canes Venatici, the recalculation of the absolute magnitudes of supernovæ and a redetermination of the Hubble constant of expansion of the universe. The radio astronomy programme at Toronto is varied in nature. It includes the measurement of absolute fluxes of the Sun and of selected sky points, and thermal sources are being developed to provide the calibration of the radiometer.

Several Divisions of the National Research Council are pursuing research on astronomical and related problems. Meteor work has been in progress for many years, and both visual and radio techniques are used. A photographic programme of meteor spectroscopy has been undertaken. Auroral and upper atmosphere research is also in progress. Work in radio astronomy includes regular observations of the total flux of radiation from the solar disk and the measurement of the absolute fluxes from bright radio sources. The existing radio astronomy site near Ottawa is now becoming unsatisfactory because of the large amount of interference arising from the expansion of the city. This has led to the establishment of a new quiet site for radio astronomy in Algonquin Provincial Park at Lake Traverse. Radio astronomical and geophysical investigations are also undertaken at several establishments of the Defence Research Board; most of this work is directed to studies of solar–terrestrial relations, the ionosphere and the aurora.

At the Dominion Astrophysical Observatory at Victoria many astrophysical investigations are in progress. Much time has been given to observations of eclipsing binaries in which one star is a cool supergiant and the companion a smaller hot star. The eclipses last many weeks, and observations at ingress and egress enable the atmospheres of the super-giant components to be studied. Five such binaries have been observed. The outer atmospheres of the supergiants contain local condensations of matter in violent mass motion, with velocities of 20 km./sec., or more, relative to the surface. The super-giants have radii, masses and luminosities of respectively 200, 20 and 3,000 times the corresponding solar quantities. Other cool stars are being observed and their spectra analysed by conventional methods. The hot *O*-type and Wolf-Rayet stars are being analysed. They also show large mass motions in their atmospheres; their atmospheres tend to be unstable, showing shells or halos, and it is likely that matter is ejected from these stars. Variable stars of the β Cephei type are being followed in detail, and cometary spectra are obtained whenever an opportunity offers itself. Much effort at Victoria is devoted to stellar motions. The radial velocities, absolute magnitudes and distances of many early-type stars are being obtained, both for individual stars and for members of clusters and associations. Several long-term programmes of radial velocity observations are also under way. New instruments under construction

include a fast grating spectrograph for the 72-in. telescope and a microphotometer recording directly the intensities of spectrum lines, and a Coudé spectrograph for the new 48-in. reflector is being designed.

An astronomical programme at the University of Western Ontario deals with the photometry and spectrophotometry of peculiar *A* stars. Observations are being made to determine which of these stars are variable and to study the nature of the light variability. The variations in their spectra are also being studied. A programme of laboratory work on molecular spectra has included the measurement of intensities in many astrophysically important bands, the photography of many bands and the determination of lifetimes of various states. An experimental study of the physical basis of the Hanbury Brown–Twiss intensity interferometer has been undertaken, and further work on this is in progress.

R. H. Garstang

THE ROCKEFELLER FOUNDATION

THE President's review from the Rockefeller Foundation annual report for 1959 covers a year of appropriations totalling more than 34 million dollars; 6,769,070 dollars went to programmes in the agricultural sciences, 12,300,071 dollars to those in medical and natural sciences and 4,049,350 dollars to those in the social sciences, while 291 Fellows from 39 countries began advanced study outside their own countries, and 339 other Fellows continued work through earlier awards, besides briefly dealing with the Foundation's operating programmes. Five main topics are discussed. The India International Centre, established at New Delhi in March 1959, with the support of 35 of the 38 universities in India, as well as Government support, is intended to provide a forum for fruitful cultural and intellectual exchange in a congenial setting where foreign and Indian leaders of thought may live together: it should serve as a place where scholars and scientists from Indian universities can meet and live with foreign cultural leaders and provide for more frequent contacts and exchange among the widely scattered Indian universities. A small publishing programme is also planned. Besides a grant of 710,200 dollars for building and selected expenses the Foundation has made allocations of 123,935 dollars for five years towards the operating expenses of the Centre.

To assist the New York University to meet the national needs for research and training defined by the Brooklyn Museum conference as necessary to ensure adequate standards of conservation of the nation's immense art heritage, the Rockefeller Foundation has provided 500,000 dollars towards the initial expenses of establishing a laboratory at the University's Institute of Fine Arts new quarters and, on a declining basis during ten years, towards certain operating expenses. The Foundation has already awarded fellowships to three members of the staff of the All-India Institute of Medical Sciences, in which, it is estimated, the Government of India will have invested the equivalent of more than 9 million dollars by March 1961, as well as six travel grants to members of the faculty and administrators since 1957, and appropriations totalling 300,000 dollars for teaching and research equipment. Still more recently, some 500,000 dollars have been released to meet foreign exchange requirements for the construction of the hospital clinic complex. At September 1, 1959, 50 postgraduate students were enrolled, of whom 40 (selected from 60 applicants) were preparing for teaching and research in the basic medical sciences, but only 13 out of 94 applicants could be accepted for postgraduate studies in the clinical fields. There are now 215 undergraduate students, and the first group will complete the basic course of study at the end of 1960.

Dean Rusk also directs attention to the unexpected dividends on virtually a world-wide basis which the Foundation's modest agricultural programme, began in Mexico in 1943, is now paying. When the project has been completed, descriptions will have been published of virtually all the races of maize in Latin America and seeds of all races will be in cold storage in three centres in Latin America with duplicate samples in storage in the United States. The seed of these maize races is available to corn breeders in all parts of the world, and the collections, which are serving as models for similar banks involving other crop plants, have been drawn upon extensively and have been widely used by maize geneticists in the United States in connexion with analyses of genetic systems of the maize plant, while classification of the living races of maize has permitted identification of many varieties in the prehistoric collection of maize from archæological sites. The Foundation has also joined forces with the Ford Foundation in establishing an international research institution to meet the urgent need for rice improvement, and, in co-operation also with the Government of the Philippines, an International Rice Research Institute is being planned on a site adjacent to the College of Agriculture of the University of the Philippines at Los Baños, towards which the Ford Foundation has appropriated 6·9 million dollars for construction and equipment and the Rockefeller 160,000 dollars for operating costs in 1960; the Institute should be in full operation by the end of 1961.

Besides this major advance, the operating programme in agriculture in 1959 saw the inauguration of the Inter-American Food Crop Improvement programme, and as a result in the Central American Corn Improvement Project there are now in the six countries concerned 23 full-time specialists instead of 5 part-time. Development of centres for fundamental research to increase knowledge of varietal improvement, soil fertility, cultural practices and the control of plant diseases is being encouraged. Besides referring to progress in the Mexican, Columbian, Indian and Chilean Agricultural Programmes, Dean Rusk emphasizes the virus research programme of the Foundation for which 1,194,640 dollars was appropriated in 1959, under which 110 agents known or suspected of being arthropod-borne are being investigated, 60 of which fall into four major groups, and very good evidence is available that 84 are transmitted by mosquitoes.

It is becoming increasingly evident that infection of man with an arthropod virus is usually accidental,

and the great problem in the temperate zones is to explain how the arthropod-borne viruses are maintained during the colder months when active insect vectors are scarce or absent. Recent investigations have shown that the young forms of several species of ticks parasitize birds, and it is thus possible that an infected tick could be transported over long distances by migrating birds. The extent and distribution of infections of man with the arthropod viruses can be determined by antibody survey work, and results of a study of residents of Athens provided evidence that the antibodies persist for at least 30 years following infection. Very valuable information has been obtained in extensive antibody surveys in the Caribbean region and a surprising finding of these immunity surveys is the wide distribution and high rate of infection with some of the recently discovered viruses.

BRITISH AGARICS AND BOLETI

A RECENT check-list of British agarics and boleti* is a logical development from the revised list (1948) of Pearson and Dennis, and embodies a number of suggestions that were put forward in that work. Many unjustified names have been dropped, and a number of new records for Britain have been added. The system used follows in the main that of Singer (1949), thus being narrower than that used in the earlier list. The work is not just the compilation that the title suggests; there are 536 pages in all, including the ancillary accounts, and the work has involved a critical study of actual material of almost three-quarters of the species listed.

Part 1*A* is a systematic list of genera that will be of assistance to foray secretaries and others who make lists of collections. Part 1*B* is the check-list proper—an alphabetical list of genera and species with synonyms, misdeterminations, authorities and references. Part 2 is a list of epithets of specific, varietal and formal rank that have been used in the past, and will be of value in translating old names in terms of the present list. Two papers published in association with the list (Part 3 by P. D. Orton, and Part 4 by F. B. Hora; *Trans. Brit. Mycol. Soc.*, **43**, Pt. 2) set out a discussion of taxonomic principles which justifies the treatment given. The view is put forward that genera and species must be accurately fixed as soon as possible, but that as yet we have insufficient information on which to erect varieties and forms. Much useful advice to collectors and taxonomists is given here. Comments on the genera used in the list are given; there is a summary of changes in genera and their subdivisions; validations of new combinations and descriptions of new species are found; and there are critical notes and keys to help understand and use the system.

The work is a massive and comprehensive undertaking, and the authors are to be congratulated on their foresight in visualizing it and on their energy in carrying it to completion. It would be strange if all mycologists were to agree with the decisions taken here: there will no doubt be those who will complain at the changes. But in the long run the sooner a stable and workable system is reached the better. This work is a major step in this direction.

D. Park

* New Check List of British Agarics and Boleti. By R. W. G. Dennis, P. D. Orton and F. B. Hora. Supplement (1960) to *Transactions of the British Mycological Society*.

ENDEMIC AND EPIDEMIC GOITRE IN ITALY

THE almost legendary disease of epidemic goitre re-appeared in 1940 in the Italian Alps and Piedmont, attacking thousands of soldiers in the Province of Cuneo, and in subsequent years spreading to the civil population of the other provinces of Piedmont. It died out in this part of Italy in 1945, but continued to flare up in small foci in Lombardy, Liguria, Emilia, Tuscany and Venetia up to 1948 (*World Health Org.*, **14**, No. 8; 1960).

The epidemic appeared in an area where endemic goitre had existed for a long time, raged for several successive years from spring to autumn, affected groups or individuals coming from outside the epidemic areas, and spread to regions from which endemic goitre had disappeared decades before.

The Italian epidemic of 1945–48 affected not only man but also dogs and pigs in some localities. At Monferrato it was shown that goitre appeared both in individuals drinking water from the mains and in those drawing their water from wells and tanks. Most of the goitres disappeared after some months, but some persisted and a few were still visible after some years. Administration of iodine brought no improvement and had no prophylactic effect.

Most of the authors who have studied the Piedmont epidemic admit a relationship between epidemic and endemic goitre. This relationship, however, has been questioned in the case of Tuscany, where epidemics without endemic goitre have been known. An analysis of recent data and of the data available on successive epidemics from the year 1700 have led A. Costa and M. Mortara to the conclusion that the two forms of goitre are diagnostically identical. They emphasize, in particular, the cerebral symptomatology that is clinically manifest to a greater or lesser extent in the two forms, and are inclined to the view that the central nervous system is affected, with elective, but not exclusive, localization in the autonomic centres in the diencephalon. Costa and Mortara consider that the cerebral phenomena manifested in acute epidemic goitre might provide some clues to the cerebral changes that result in the cretinism and deaf-muteness of endemic goitre. An important fact is that endemic cretinism is still to be found in its old sites—the Alps and the southern Apennines, including several surrounding hill and plain areas—but does not appear in association with endemic goitre in the south of Italy. Cretins are rare in the

new generations, and among them myxœdema and dwarfism due to thyroid insufficiency are less frequent.

War difficulties and the comparative lack of knowledge about the symptoms of thyroid dysfunction impeded research at the time of the Piedmont epidemics. Most of the tests were normal. Neutropenia was observed with lymphocystosis, and the blood iodine level was high. No serum antibody to human thyroglobulin was found.

In the Piedmont the uptake of radioiodine is generally greater than normal in the mountain, hill and plains foci of endemic goitre, and the iodine taken up is organically fixed in the thyroid. The excretion curve of radioiodine is slow in comparison with that in hyper-thyroidism. Thyroid clearance of iodine is high, kidney clearance is normal. Body growth, bone maturation, and sexual development in children are the same in endemic and non-endemic areas.

During the epidemics of goitre in the endemic area, the environmental factors traditionally considered to influence the disease—water, malnutrition, deficiencies, consanguinity, and shortage of iodine—all received attention. But none of these factors seems to be responsible for endemic goitre or for the Piedmont epidemics. The epidemic spread gave rise to the hypothesis of an infectious origin, with transmission by an intermediate host favoured by hot weather —for the epidemics occurred mainly in summer. In Umbria, the heavy consumption of *Brassicæ* containing goitrogenic factors, and in mountain areas a diet lacking in proteins and in some essential amino-acids were blamed. Endemic goitre is essentially rural in character, and Costa and Mortara consider that its frequent occurrence in localities where sheep are raised in stables may be more than a simple coincidence. The possible influence of radiation has not been ruled out, but radon has been sought in the atmosphere and in the water, and atmospheric radioactivity has been measured, without any relationship with endemic goitre being found. The iodine content of air and water and the excretion rate of iodine have shown no significant differences between endemic and non-endemic areas.

Goitre is not a static disease in Italy. It was widespread in the last century and the first decade of this century; then its prevalence decreased. It appears now that the descending curve has been arrested. Goitre has become more common in central and southern Italy, and has flared up in localities of northern Italy which had for some time appeared free from it. In certain areas the epidemics seem to have left endemic goitre in their train. The phenomenon of epidemic goitre in Italy has given rise to observations and research that are not paralleled in other countries; but so far its origin remains unexplained.

SOME CHARACTERISTICS OF GEOMAGNETIC MICROPULSATIONS (*Pc*)

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A DETAILED investigation[1] has been carried out on geomagnetic micropulsations recorded by rapid-run magnetograms during the International Geophysical Year (1957–1958), from seventeen observatories as widely distributed geographically as possible (see Table 1). The results from these seventeen observatories were recorded photographically, not only on rapid-run magnetograms with a recording speed of 20 mm. per 5 min., but also on standard speed magnetograms with a recording speed of 20 mm. per hr.

Micropulsations (*Pc*) are defined as continuous pulsations usually appearing on the daylight side of the Earth, lasting for many hours with periods in the range of 10–60 sec., and amplitudes of the order of a few tenths of a gamma in middle latitudes. However, if the evidence of all investigators over a wide area is considered, it becomes apparent that these micropulsations do not correspond to a single wave-band but to two wave-bands, the first a shorter-period micropulsation (about 15–30 sec.) and the second a longer-period micropulsation (about 30–90 sec.). Fig. 1 shows the spectrum of micropulsations which have been investigated by several workers from stations all over the world, and clearly illustrates the division into these two different wave-bands.

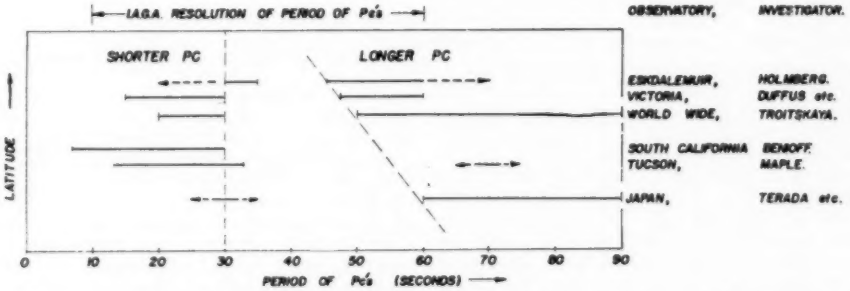

Fig. 1. Latitudinal distribution of spectrum of micropulsations (*Pc*) with periods greater than 10 sec. as reported by different investigators from data shown on the right-hand side

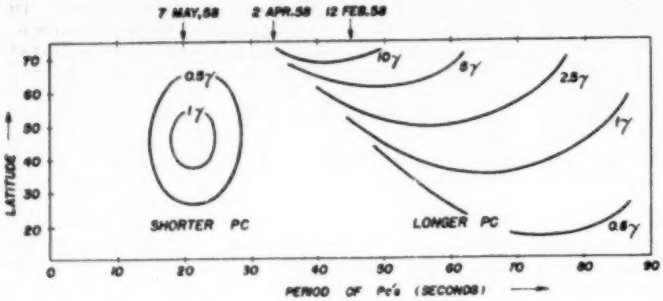

Fig. 2. Equi-amplitude contours of micropulsations (*Pc*), derived from Fig. 1

Typical of longer-period micropulsations were those observed on February 12 and April 2, 1958, which have periods of 33 and 45 sec., respectively. These examples have coherent, synchronous wave-forms over a wide area. It is also worth noting that micropulsations of this type often have amplitudes up to ten gammas or more in polar regions.

A pulsation appeared on May 7, 1958, with a period of about 20 sec. and with maximum amplitude at about lat. 50°. Many similar examples (such as those which occurred on February 8 and May 28, 1958) have been found: they all have in common comparatively short periods (from about 15 sec. to 30 sec.). Those shorter-period micropulsations show different periods at different latitudes, although it is not possible to be definite on this point since the speed of the rapid-run recordings is too slow for any fine resolution.

Combining the results of previous investigations with the distribution in latitude of the amplitude of micropulsations which has been derived in the present analysis, equi-amplitude contours can be drawn as shown in Fig. 2. The different distribution in latitude of the amplitude of the two wave-bands of micropulsations and the geographical variation of period of the shorter micropulsations lead to the conclusion that the longer and shorter period micropulsations are probably caused by hydromagnetic waves in the upper and lower parts of the outer atmosphere respectively.

Between about 2300 and 2400 U.T. on February 12, a series of very intense (longer-period) micropulsations were observed. They continued for about 1 hr. and appear as thick traces on ordinary magnetograms because of the slow paper speed (see Fig. 3). Of special interest are the pre- and post-oscillations of this particular micropulsation. The figure shows clearly the intense pulsation of about 6-min. period which prevailed during the previous 2 hr. and after the micropulsation. Usually this type of pulsation is observed in polar regions and is frequently associated with micropulsations. They are nearly synchronous over a very wide area, from the Arctic to the Antarctic, and on occasions appear in equatorial regions as well. Moreover, the polarization is nearly linear at each station. This type of pulsation has been designated a long-period continuous pulsation (*LPc*).

Sometimes beating oscillations of micropulsations, which appear mainly in middle latitudes, occur simultaneously with these linear pulsations in polar regions. The periods of both the beating micropulsations (*Pc*) and the pulsations (*LPc*) are about the same, namely, 3–7 min. (ref. 2). Moreover, the ideal equivalent overhead current-systems which have been drawn for the examples on February 12 are

Table 1. Stations supplying Observations used in this Report

Station	Abbreviation	Geographical Lat.	Geographical Long.	Geomagnetic Lat.	Geomagnetic Long.
Point Barrow	*PB*	71° 18′ N.	156° 46′ W.	68·6° N.	241·0° E.
College	*Co*	64° 51′ N.	147° 50′ W.	64·7° N.	256·5° E.
Big Delta	*BD*	64° 09′ N.	145° 51′ W.	64·4° N.	259·0° E.
Healy	*Hy*	63° 51′ N.	149° 00′ W.	63·6° N.	256·5° E.
Sitka	*Si*	57° 03′ N.	135° 20′ W.	60·0° N.	275·4° E.
Lovo	*Lo*	59° 21′ N.	17° 50′ E.	58·2° N.	105·8° E.
Fredericksburg	*Fr*	38° 12′ N.	77° 22′ W.	49·6° N.	349·9° E.
Tucson	*Tu*	32° 14′ N.	110° 57′ W.	40·4° N.	312·1° E.
Kakioka	*Ka*	36° 14′ N.	140° 11′ E.	26·0° N.	206·0° E.
Honolulu	*Ho*	21° 18′ N.	158° 08′ W.	21·0° N.	266·4° E.
Guam	*Gu*	13° 27′ N.	144° 45′ E.	3·9° N.	212·8° E.
Koror	*Kr*	07° 16′ N.	134° 32′ E.	3·3° S.	203·5° E.
Apia	*Ap*	13° 48′ S.	171° 46′ W.	16·0° S.	260·2° E.
Hermanus	*Hr*	34° 24′ S.	19° 13′ E.	33·3° S.	80·3° E.
Byrd Station	*By*	79° 59′ S.	120° 01′ W.	70·6° S.	336·0° E.
Little America	*LA*	78° 18′ S.	162° 10′ W.	74·0° S.	312·0° E.
Wilkes	*Wk*	66° 15′ S.	110° 31′ E.	77·8° S.	179·0° E.

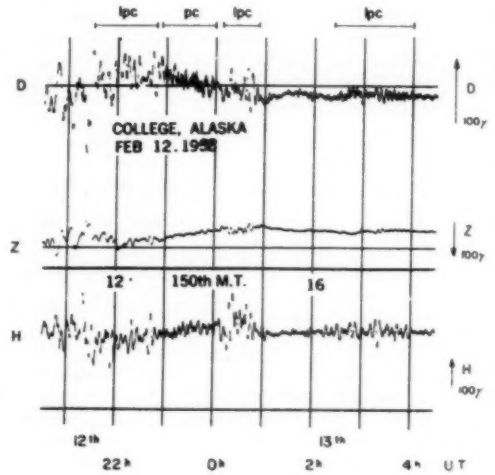

Fig. 3. Examples of micropulsations (*Pc*) and long-period continuous pulsations (*LPc*). Traces are from ordinary magnetograms at College and Fredericksburg on February 12–13, 1958

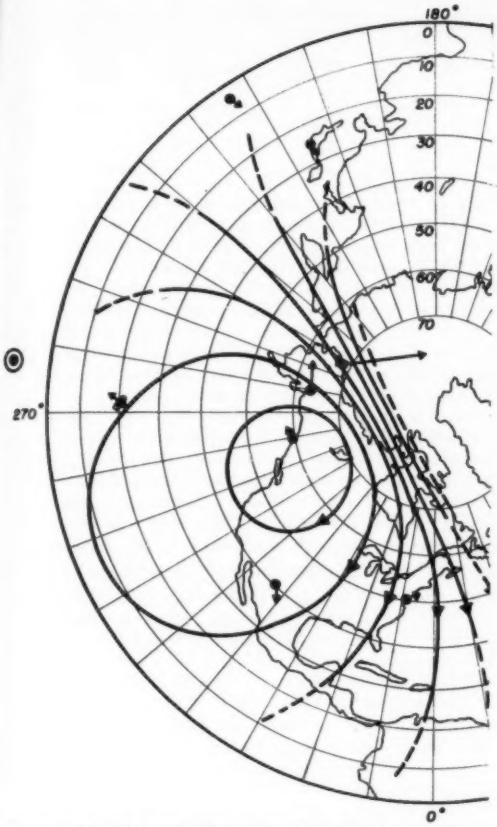

Fig. 4. Equivalent overhead current system for micropulsations (*Pc*) at 2345–2350 U.T. February 12, 1958. Electric current between successive lines is 5×10^3 amp.

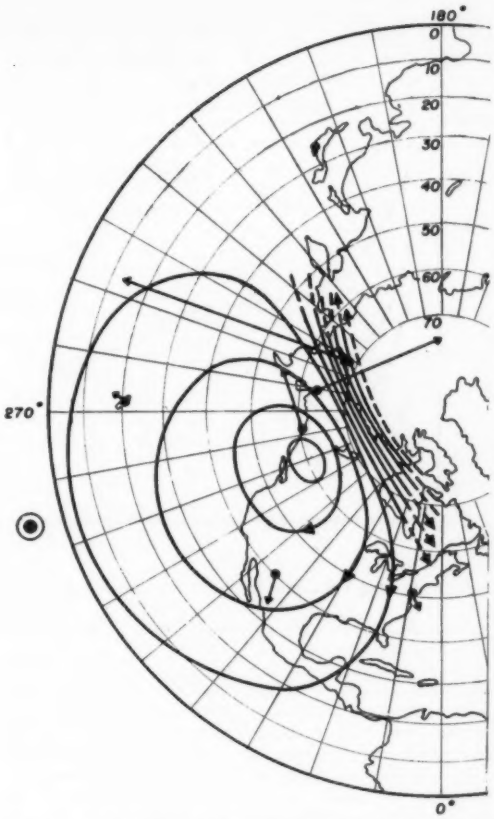

Fig. 5. Equivalent overhead current system for long-period continuous pulsations (*LPc*) at 2215–2230 U.T. February 12, 1958. Electric current between successive lines is 5×10^4 amp.

strikingly similar for both except in the polar region (see Figs. 4 and 5). The close relations between them indicate that both pulsations have a common source, probably outer atmospheric oscillations.

We wish to acknowledge the sources of data from the various geomagnetic stations and the International Geophysical Year World Data Centre *A*. The research was supported by the Geophysics Research Directorate, Air Force Cambridge Research Center.

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SMALL-ANGLE X-RAY SCATTERING BY PILE-IRRADIATED GRAPHITE

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PREVIOUS attempts to study the nature of radiation damage in graphite by small-angle X-ray diffraction are reported as being unsuccessful[1]. This was because the scattering due to radiation-induced defects was small in comparison with that due to defects already present before irradiation. The purpose of the present communication is to report that small-angle scattering of intensity much greater than that found with unirradiated graphite has been observed with specimens of polycrystalline artificial graphite of reactor grade heavily irradiated (*Dido* doses 5×10^{20}–17×10^{20} n.v.t. measured thermal flux) at temperatures in the vicinity of 150° C.

The specimens were in the form of solid half-cylinders and were examined in reflexion using a Philips 1050 diffractometer and nickel-filtered copper $K\alpha$ radiation. With $\frac{1}{6}$° divergence slits the minimum scattering angle (2θ) at which observations could be made was 3°. All the measurements were made under identical conditions. The variation of scattered intensity with 2θ is shown in Fig. 1 for three irradiated specimens and a typical unirradiated specimen. A fourth specimen, irradiated at 300° C., showed no

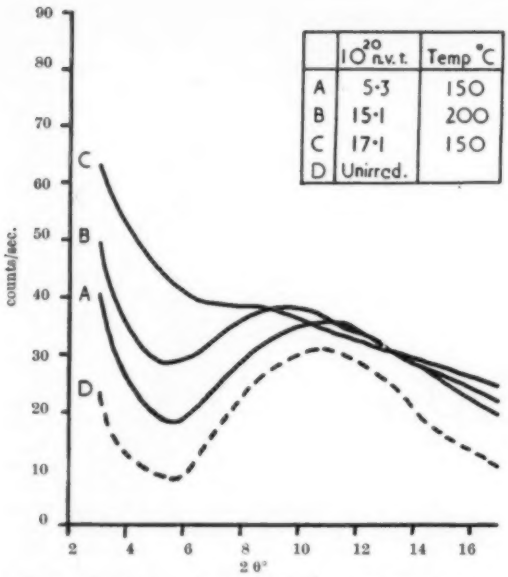

Fig. 1. Low-angle X-ray scattering from irradiated graphite

increase in small-angle scattering within the observable range of 2θ.

The component of the intensity attributable to radiation damage was obtained by subtracting from each irradiated intensity distribution the intensity distribution of an unirradiated specimen cut from the same block of graphite in the same orientation relative to the direction of extrusion. This procedure compensates for slight block-to-block variations in the pre-irradiation small-angle scattering and also for variations in the degree of preferred orientation. The latter is manifested as differences in the height of the diffuse peak in the vicinity of $2\theta = 11°$ which is due to the short wave-length 'white' X-rays reflected by the 0002 graphite lattice planes. Radiation

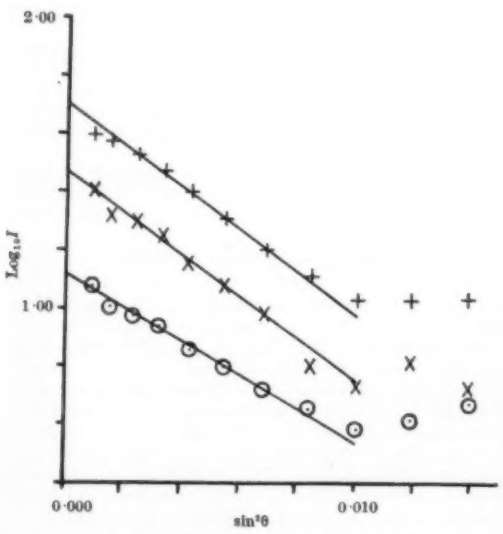

Fig. 2. Slope analysis (Table 1: *A*, ○; *B*, ×; *C*, +)

Table 1

Specimen	Dose (n.v.t. × 10^{20})	Temperature of irradiation (°C.)	$\bar{R}$ (Å.)	R_{disk} (Å.)	N (per cent)	N (per cent) / (n.v.t. × 10^{20})
A	5·3	150	2·46	3·5	4·3	8
C	17·1	150	2·77	3·9	13·2	7·7
B	15·1	200	2·73	3·9	8·9	5·9

damage results in broadening of the crystalline X-ray reflexions, but in the case of this 'white' radiation 0002 peak the change is very slight and introduces a negligible error into the small-angle scattering curves derived by the subtraction procedure. However, radiation broadening of the copper $K\alpha 0002$ peak in the vicinity of 25° results in major differences which extend as low as $2\theta = 11°$, and as the subtraction procedure cannot compensate for this the curves are in error above this value of 2θ.

The slope-analysis method was applied to the scattering distributions thus derived without making specific corrections for vertical divergence[2], and it can be seen from Fig. 2 that the plots of log I versus $\sin^2\theta$ are good approximations to straight lines up to $\sin^2\theta = 0{\cdot}009$ ($2\theta = 11°$). The slopes of these plots give the average 'radius of gyration' $\bar{R}$ of the scattering centres. Values of $\bar{R}$ are listed in Table 1 together with values of $R_{disk} = \bar{R}\sqrt{2}$. R_{disk} is the average radius of the scattering centres if they are assumed to be disks of a thickness much less than their diameter. The intensity distributions are not sufficiently accurate to give any reliable indication of the shape of the scattering centres; but it is likely that in irradiated graphite they are disk-like aggregates of interstitial atoms, sandwiched between the graphite layers and formed by clustering of the atoms displaced by neutron bombardment, as already suggested[1].

Interpretation of the present results in terms of this picture presupposes that the observed scattering is entirely due to the clusters of interstitials, and neglects any contribution from the vacancies which must exist in comparable numbers. This is justifiable in that single interstitials probably have a far greater degree of mobility than the vacancies at the temperature of irradiation, and are therefore more likely to form clusters under the influence of the strain fields set up in the adjacent graphite layers. Such clusters of ten or more atoms would have a small-angle scattering power much larger than that of the more numerous single vacancies since the small-angle scattering power of an individual centre is proportional to the square of the number of electrons it contains.

The log I − $\sin^2\theta$ graphs show little decrease in slope with increase in $\sin^2\theta$, a feature which normally indicates that the scattering centres are all much the same size. In the present case one would expect a range of sizes, but perhaps there is some feature of the mechanism of formation which leads to one size being more probable than any other under given conditions. This size as characterized by R_{disk} increases with dose.

The supposition that the scattering centres are disks of interstitials enables an estimate of their concentration to be made from the absolute magnitude of the total intensity scattered by them, which in the present case was measured by comparison with the integrated intensity of the 002 reflexion of finely powdered rock-salt. The concentration of interstitials, expressed as a fraction N of the number of atoms per unit volume in the perfect graphite lattice, is

related to the total scattered intensity $I_{tot.}$ by an equation of the form:

$$I_{tot.} = KN\alpha R_{disk}$$

where R_{disk} is the value of the radius given by the slope analysis, α is the number of interstitials/unit area of disk and K is a constant in which are combined various physical and instrumental factors. The value given to α was 0·2 interstitial/Å.^{2}, which corresponds to occupation of one in every two interstitial sites within the periphery of a disk, that is, an average distance of 2·44 Å. between adjacent interstitials. If a higher density is assumed, for example, occupation of every site, the interstitials would be 1·45 Å. apart and would have to be graphitized to a high degree.

The values of N are given in Table 1 together with the number of interstitials/10^{20} n.v.t., which agrees closely with estimates made by other experimental and theoretical methods[3]. It is perhaps noteworthy that the specimen irradiated at 200° C. shows a lower value of N/n.v.t. than those irradiated at 150° C., a point which it is intended to verify in further experiments.

So far it has been tacitly assumed that the scattering observed here is true small-angle scattering. In several instances supposed small-angle scattering from inhomogeneities in a crystal lattice has since been shown to be multiple Bragg reflexion from distorted lattice planes[4]. Though it is difficult to perform quantitative calculations on this type of multiple reflexion it seems that the intensities observed in the present experiments are at least two orders of magnitude larger than the maximum possible by multiple reflexion. Further, neutron-diffraction experiments[5] in which the wave-length employed was such as to place all reflexions from lattice planes beyond the cut-off, that is, $\sin\theta > 1$, definitely indicated the presence of point defects in graphite irradiated to a level comparable with the present specimens. Owing to the difficulties inherent in neutron diffraction, these experiments did not show whether or not the defects were clustered, but the number of defects detected agrees reasonably well with the present ones. Thus it can be argued that neutron diffraction shows that point defects occur in irradiated graphite in sufficient concentration to cause true small-angle X-ray scattering provided they cluster in a manner similar to that postulated here. The observed small-angle scattering interpreted in this way gives a reasonably self-consistent picture which is also consistent with theoretical calculations and other types of experiment.

This work will be continued, and it is hoped that in future the use of plane-polarized X-rays in which the plane of polarization can be rotated[6] will permit an experimental distinction to be drawn between true small-angle scattering and multiple Bragg reflexion.

I am indebted to the Head of the United Kingdom Atomic Energy Authority's Culcheth Laboratories where this work was carried out, and to the Director of the Central Electricity Generating Board Berkeley Nuclear Laboratories, for permission to publish this article. I also thank Drs. A. L. Sutton and G. K. Williamson for helpful discussions and advice.

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SUCROSE CRYSTALS

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Tate and Lyle, Ltd., Thames Refinery, London, E.16

Layer Growth

In an earlier article[1], I was able to report having found that sucrose crystals grew layer-wise, just as Drs. Bunn and Emmett had previously described the growth mechanism of crystals of many salts. I am now able to report that a sucrose crystal immersed in a very slightly sub-saturated solution dissolves by a receding layer mechanism. In a growth cell under the light microscope, one may vary the degree of saturation of the mother syrup by temperature control, and by this means produce advancing or receding layer fronts at will. If a crystal is immersed in very slightly sub-saturated syrup, faint etch pits form first. Later, as the natural gradient of exhaustion of the contact layer becomes established, the receding layers become visible, first near edges and corners, then spreading over the whole crystal surface, and even in the etch pits. I have been able to record this, both by photo-micrography and by ciné-micrography.

Drs. Dunning and Albon[2] have reported spiral growth in sucrose crystals nucleated and grown under laboratory conditions, but have not hitherto been able to observe them in commercially grown crystals (private communication). Having now observed a large number of growing and dissolving crystals under the microscope, my impression is that certainly in crystals which have been grown under the practical conditions of varying supersaturation and occasional sub-saturation the layer fronts are so strongly developed as to continue to efface spirals by overgrowth, just as they overgrow pockets of solution in making fluid inclusions. I feel that spirals and layers should be regarded as two distinct, and not necessarily connected, mechanisms by which crystal growth may take place. The latter become more strongly developed with increasing crystal size, also after partial dissolution or mechanical damage. It is becoming increasingly evident that, even on an apparently dry sucrose crystal, the surface should be regarded as in a state of dynamic, not static, equilibrium.

Thin Films

Crystallization in thin films (up to a hundred or so microns in thickness) exhibits different phenomena, according to the nature of the bounding media. These may conveniently be grouped in three cate-

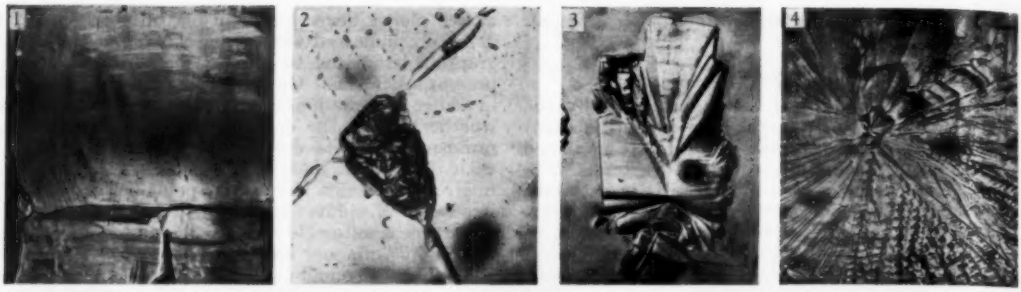

Fig. 1. Mild dissolution: etch pits and receding layers
Fig. 2. Drainage system at 3·5 supersaturation. Mass/nucleation hazard, also how spherulitic crystals commence exploring growth
Fig. 3. Disordered crystal at 2·4 supersaturation; note spherulitic eruptions
Fig. 4. Radialite from film, showing part-rhythmic growth
Fig. 5. An example of rhythmic dendritic exploring growth
Fig. 6. Sodium chloride grown in vapour-pressure controlled film; whisker, acicular, tabular, etc., forms
Fig. 7. Radialitic carpet of sucrose from film only one or two microns thick. Note curvature

gories; (*a*) glass or plastic (relatively inert); thus glass/syrup/glass; (*b*) atmosphere with controlled humidity (causing migration of water molecules to or from the solution); thus glass/syrup/air; (*c*) sucrose crystal (offering or receiving sucrose molecules to or from the solution); thus crystal/syrup/air.

(*a*) The first system has very wide application to problems of form, rate of growth, etc. By adopting techniques on the lines of microbiological work one may avoid intrusion of external nuclei. By warming a supersaturated solution to the temperature of saturation before using, one may seed a film of the syrup, by means of a needle dipped in specially prepared nuclei, without producing fresh nucleation by mechanical hazard. Probably the most challenging demonstration by this system is that of the greatly varying rate of growth of different nuclei when introduced into the same medium. By seeding with two needles carrying different types of nuclei, and with one drawing a horizontal and the other a vertical line, then 'incubating', any difference in the natural rate of growth of the two types of nuclei should be revealed. By this means it appeared that particles of pulverized crystal at first grew more rapidly than whole seed crystals, until the regular crystal form had taken shape. On extending the growth period, however, there appeared far more difference in size between neighbouring crystals of the same origin than appeared overall between those from the different origins.

Another application is to the study of the effect of various impurities on crystal form, for example, increasing proportion of raffinose, which produces first a lengthening in the axis ratio, then striking wedge-shaped forms.

Still another application is to study whether crystals can display any form of 'inheritance'. Can a seed crystal, produced in circumstances favouring a characteristic form, carry forward the special feature if transferred to a different medium? I have tested this by transferring crushed portions of the spherulitic form produced at high supersaturation to solutions of low supersaturation. The broken edges were repaired, but growth was so slow that normal nuclei, developed in the new medium, rapidly outgrew the spherulitic fragments. An example of internal rather than external specialized form is that reported by Drs. Dunning and Albon[2], who found spiral growth on crystals nucleated and grown in sucrose solution containing up to 1 per cent of dextrose to have a highly significant heightening of the step as compared with that in pure sucrose solution, hence probably an increased growth-rate. I have not been able by the above means to satisfy myself with evidence of effective 'inheritance' in this case.

By applying non-sucrose components to one exposed edge of an '*a*' type film, after seeding, diffusion may reveal the effect of the component thus introduced on the resulting crystallization, as a prelude to a more controlled investigation.

(*b*) The glass/syrup/air system offers a remarkable facility for attaining and maintaining any desired degree of supersaturation without the handicap of virtually uncontrollable nucleation in the higher range. It also renders possible the production of controlled crystallization in films of thickness down to the millimicron range.

To eliminate rogue nucleation, the solution should be sub-saturated when placed on the slide. Extremely thin films may be obtained by dilution and drainage. The prepared slide is placed in a desiccator, controlled by means of moist salts, glycerine, etc. If the desiccator is suitably constructed, both observation and photo-micrography may be made *in situ*.

Both the natural hazard of nucleation and the resulting crystal form are very strikingly affected by

the degree of supersaturation. With sucrose, nucleation is maximal at about 3 supersaturation, at which stage perhaps one nucleus may form per 24-hr. at 20° C. in a 0·3 gm. film. At less than 1·5 and greater than 5·0 supersaturation, weeks may elapse without a single nucleus forming naturally. Supersaturation has been expressed in the form recommended by the International Commission for Uniform Methods of Sugar Analysis, 1958, namely :

$$\frac{\text{gm. sucrose/100 gm. water at temperature } t}{\text{gm. sucrose/100 gm. water in saturated solution at temperature } t}$$

Regarding the influence of supersaturation on crystal form, at the 1·1 level normal to industrial production, euhedral crystals form. At 1·5 signs of disorder appear, this increasing as supersaturation increases. At 2·5 supersaturation spherulitic multi-crystals appear, together with some abnormal mono-crystals, which by this stage show occasional eruptions of multi-crystal segments, frequently at rhythmic spacing on the mono-crystal. By 4·0 supersaturation practically all nuclei are spherulitic. When grown in a thin film the spherulitic nucleus develops two-dimensionally, that is, 'radialitically', this proving most illuminating on details of structure. Normally, adjoining ray crystals are in harmony of orientation, as with a fan, but the full 360° may include several such groupings, each of different orientation. I have recorded layer growth, also hour-glass and veil inclusions on radialite crystals, just as with normal mono-crystal forms[3]. My impression is that all these phenomena, culminating in spherulitic nucleation, are the outcome of the build-up of the exothermic heat of crystallization even to the point of local fusion. Mosaic may result from one form of the above disorder.

If the film has been gelled with agar or gelatine the spherulitic nucleation occurs even as low as supersaturation 1·6, and two easily recognized variant forms ensue. With agar, sharply contoured layer growth scales abound; whereas with gelatine, multitudes of gentler curves take the place of the more normal rectilinear radialites.

If crystals are allowed to grow in a thin layer of solution in a covered Petrie dish exposed to atmosphere of 50–60 relative humidity, the upper surfaces of the crystals become exposed. Surface forces cause solution to be drawn up and continue crystallization, until the residual film is so thin that this flow ceases. Alternatively, one may commence with a film of similar thinness and permit nucleation therein. In either case, the formed crystal then develops an 'exploring growth'—reminiscent of *Amoeba*—which is truly delightful in its rhythmic beauty. Familiar contour angles may be recognized, repeating in nodal and anti-nodal intensity, strongly reminiscent of Liesegang precipitation. A little reflexion on the manner of growth will indicate that each complete pattern, though apparently so complex, is yet as truly a mono-crystal as any simple euhedral crystal. These truly dendritic forms normally develop rectilineally, but sometimes exhibit curvilinearity, developed by faults, to permit growth to enter a zone richer for growth. This exploring growth can proceed in films so thin as to render crystals invisible in the light microscope, and can develop crystals with truly dry surface areas. It is extremely slow, continuing for weeks.

(*c*) The crystal/syrup/air system is normally met in industrial application, and will be dealt with more fully elsewhere. It is pertinent to report here that the remarkable ability of syrup to dry almost to the amorphous and extremely slowly crystallizing form, contributes considerably to problems of bulk storage. Probably all the crystal forms described above may be formed during the granulation or drying of commercial sugar.

Though my work has been mainly with sugar, the system of crystallizing in vapour-pressure controlled films, will obviously have wide applications. Sodium chloride so treated has yielded encouraging results. Exploring growth is shown, and whisker growth appears to occur sub-surface, since I have photographed whiskers growing under other crystals. The subsequent development from whisker to needle, also of strikingly clear tabular crystals, is easy to follow and is being filmed, as are many of the sucrose phenomena described herein.

For those interested in this work, a wide and rapidly increasing range of photomicrographs is now available of the diverse forms of sucrose crystallizing under this vapour pressure control.

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[2] *Nature*, **180**, 1248 (1957).
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PROTEINS INDUCED IN PLASMA BY ŒSTROGENS

By Dr. O. A. SCHJEIDE and Dr. M. R. URIST

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THE fact that at least two—and probably several—of the proteins of egg yolk are synthesized in the liver of the hen and transferred via the blood plasma to the ovary (where they are deposited as granules in the ovum) is well established[1–3]. However, recent articles by Prof. R. H. Common and Chi-Ching Mok[4] and Joubert and Cook[5], convey the impression that much of the data available in the literature, some of it our own[1,3,7–9], has failed to come to the attention of workers interested in the elucidation of the proteins involved in avian reproduction. Further, because of the limitations of techniques employed in any single laboratory, much confusion exists with respect to the nomenclature, chemical constitution and physical orientation of these proteins. We have therefore prepared the following critical analysis.

First, the administration of œstrogen to the hen, pullet or rooster results in the amplified appearance of at least three major protein components in the

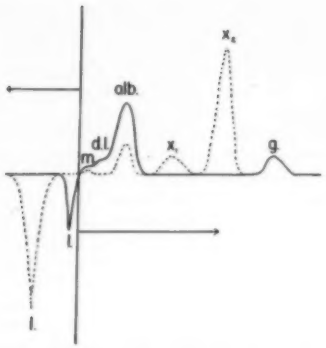

Fig. 1. Composite ultracentrifuge patterns from plasmas of control and œstrogenized birds. Areas under the peaks are equivalent to concentration. Negative peaks are due to the high proportions of lipid in such fractions. Areas defined by the solid line indicate control values. Those defined by the broken line indicate values for œstrogenized bird.
l, Light lipoprotein; *m*, mucoprotein; *d.l.*, dense lipoprotein; *alb.*, albumin; X_1, X_1-phosphoprotein; *g*, globulin; and X_2, X_2-lipoglycoprotein

plasma; there are the two mentioned by Common and Chi-Ching Mok (phosvitin and lipovitellin) and also a light lipoprotein varying in flotation-rate upwards from S_f 15 (ref. 9). (We have chosen not to apply a generic name to this protein, and have used the term X_1-phosphoprotein in place of phosvitin and the term X_2-lipoglycoprotein in place of lipovitellin, for several reasons that will become evident.)

The appearance of $X_1 - X_2$ in the serum is not the only change in the plasma of œstrogenized birds. In addition, there are absolute decreases in albumin, globulins, normal light lipoproteins (Sf 0–15), dense lipoproteins ($-S$ 0–10), and mucoproteins (ref. 9 and unpublished work). The severity of the decreases is proportional to the amount of œstrogen administered and to the increases in components induced by œstrogen. A schematic diagram of these changes as they might appear in a composite ultracentrifuge pattern of chicken plasma is presented in Fig. 1.

Secondly, the implication must be questioned that phosvitin, lipovitellin and a component referred to as γ-livetin[4] are present in the plasmas in the form of complexes with each other that are similar to the complexes formed when egg yolk or the serums of œstrogenized birds are greatly diluted with distilled water[1]. Of course, in the native egg yolk of the hen, the X_1 and X_2 proteins (plus lighter lipoproteins and a relatively small amount of nucleoprotein) are present in the form of giant complexes termed granules. (Such granules comprise approximately one-fifth of the total volume of the egg yolk, and on isolation and dissolution with 10 per cent sodium chloride have been demonstrated to contain all the X_1 and X_2 in egg yolk[3].) However, since the X_1 present in yolk granules retains a high percentage of bound calcium, the granule complex must be very different from the complex which forms when either plasma X_1 and X_2 or egg yolk X_1 and X_2 are diluted with distilled water. In these latter cases, the complex appears to result from the removal of naturally bound calcium from the X_1 phosphoprotein (this phosphoprotein contains 10 times more calcium per unit weight than does casein of milk) exposing numerous negative charges on the X_1 molecule which readily combine with the positive charges made available on the phospholipid portion of the X_2 protein. The amount of calcium associated with serum X_1 in the native state is sufficient to neutralize the negative charges of the phosphoserine in the molecule so that no complex is formed; and, indeed, phosphorus and calcium analysis of the X_2 (lipovitellin) component of serum show it to be uncontaminated with X_1. As for γ-livetin, its existence has never been demonstrated in plasma or serum.

The term γ-livetin was introduced by Joubert and Cook[5] to account for a protein component of egg yolk that, when resolved in the ultracentrifuge, trailed behind a fraction which they termed a complex of γ-livetin, phosvitin and lipovitellin (Fig. 2).

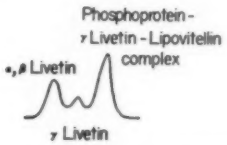

Fig. 2. Schematic sketch of egg yolk ultracentrifuge patterns as obtained and interpreted by Joubert and Cook (ref. 6)

These workers claimed that they were unable to resolve γ-livetin in quantity from this so-called complex in 5 per cent sodium chloride, but that it could be resolved in larger amounts in the presence of phosphate buffer (*p*H 7·6), barbiturate buffer (*p*H 9·0) or glycine buffer (*p*H 10·4). We have discovered that yolk X_2 lipoglycoprotein splits into smaller molecules when placed in an alkaline medium and that X_1 phosphoprotein tends to conjugate (thus sedimenting faster) in the same medium. Thus, the γ-livetin that Joubert and Cook observed was probably the alkaline breakdown product of X_2 which, according to our experience, shows a somewhat similar sedimentation-rate (approximately S 4–5) to that of X_1, in 10 per cent sodium chloride.

In work carried out at about the same time[3], we had examined the ultracentrifuge patterns of egg yolk proteins in 10 per cent sodium chloride solutions and were not able to resolve as much of the so-called γ-livetin as Joubert and Cook did in their buffered experiments. Furthermore, because of previous experience with yolk proteins of the frog[7], in which case we had isolated the trailing component by ultracentrifugation and characterized it, we had suspected that the so-called γ-livetin was actually the natural form of the phosphoprotein from which phosvitin is derived. Subsequent centrifugal isolation of this fraction in the partition cell for the preparatory ultracentrifuge and chemical characterization revealed it to be nearly pure phosphoprotein (X_1), containing approximately 10 per cent phosphorus[1] and 50 per cent serine (unpublished work), of which almost 50 per cent was phosphoserine. (Our best sample of phosvitin prepared by the method of Joubert and Cook[5] gave this same amino-acid analysis.) Furthermore, protein, calcium, phosphorus and lipid analysis of the remaining X_1 plus X_2 lipoglycoprotein fraction (see Fig. 3) revealed that no significant amount of phosphoprotein was actually complexed with the X_2 lipoglycoprotein (lipovitellin) in 10 per cent sodium chloride, nor could there have been any large amount of 'heavy X_1' present, having the same sedimentation-rate as X_2. (We use the term 'heavy X_1' to describe an, as yet, theoretical molecule having twice the molecular

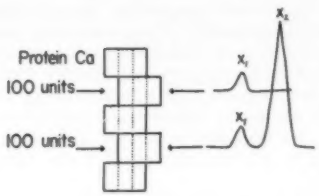

Fig. 3. Layering of proteins in partition cell for the ultracentrifuge

weight of X_1 as we have described it and consisting, in theory, of two units of the X_1 having the sedimentation constant S 4·75. Turtle egg yolk X_1-phosphoprotein appears to have approximately twice the molecular weight of hen egg yolk X_1, but its structure has not yet been determined.)

A complex of X_1 with X_2 would have increased detectably the sedimentation-rate of the X_2. The possibility of a third, fourth or nth component present as an impurity in the X_2 peak cannot be excluded, but X_1 does not seem to be one of these, and γ-livetin, if present, cannot be complexed with X_2, since it would, in such an orientation, again completely modify the sedimentation-rate of X_2.

Thirdly, from the foregoing, it is obvious that at least phosvitin as obtained by the chemical procedures of Joubert and Cook[5], or by the electrophoretic separations of McCully, Maw and Common[10], is a derived protein of a form considerably different from its physiological counterpart. In its natural state, phosvitin contains approximately 7 per cent calcium, as determined by calcium analysis of phosphoprotein (X_1) isolated ultracentrifugally from either serum or egg yolk. During the chemical or electrophoretic isolation of phosvitin, nearly all the calcium is removed. (75 per cent of the calcium associated with the phosphoprotein is irretrievably lost by a single precipitation in distilled water) and often a considerable amount of the phosphorus is removed by hydrolysis. These losses, together with a possible breaking of the phosphoprotein backbone, are reflected by a much slower sedimentation constant for egg yolk phosvitin (S 3·0) as compared with egg yolk X_1, its naturally occurring counterpart (S 4·75) and a much faster rate of migration on paper at a pH of 8·6 (ref. 10).

Lipovitellin, as prepared by Joubert and Cook[6], sediments at nearly the same rate as the naturally occurring egg yolk dense lipoprotein (X_2 isolated by ultracentrifugation); but in the chemically prepared form it is usually contaminated with both lipovitellenin and livetins. Pre-isolation of the egg yolk granules was found by us to eliminate the livetins, since these are found only in the yolk fluid. The egg yolk granules contain only phosphoprotein and dense and light lipoproteins in significant proportion, and the light lipoproteins can be floated off by ultracentrifugation. The dense lipoprotein (lipovitellin) contains a considerable amount of polysaccharide, and approximately 20 per cent of its weight is due to the presence of lipid, mainly phospholipid[3]. Many workers tend to obtain higher lipid values for this component, due to contamination with lipovitellenin (or light lipoproteins), which are co-precipitated with the phosphoprotein and lipovitellin on dilution with distilled water.

Finally, it should be emphasized that the natural form of phosphoprotein from egg yolk (X_1) is only half the size of its serum precursor as measured under our conditions, although it contains the same proportions of phosphorus and calcium[3], and the natural form of the dense lipoprotein of egg yolk (X_2) is only half the size of its serum precursor, although it contains the same proportions of total lipid and phospholipid[3]. Thus, striking alterations have taken place in the molecules during the transfer from blood to yolk granule or from yolk granule to solution. In confirmation of these changes, when aliquots of egg yolk X_1 and X_2 and serum X_1 and X_2 were mixed and centrifuged together, the ultracentrifuge pattern shown in Fig. 4 was obtained.

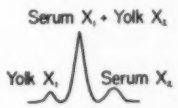

Fig. 4. Sedimentation pattern of a mixture of egg granules, proteins and serum from an œstrogenized rooster

It is mainly because we do not yet know the exact nature of these and possibly other alterations that we prefer to use the terms serum X_1 and X_2 and egg yolk X_1 and X_2. These terms both link and distinguish the four different components which may, or may not, be relatively homogeneous materials. The terms phosvitin and lipovitellin, however, refer to proteins which are known to be altered and mixed, and thus do not show due respect for the integrity of these important physiological entities.

These investigations were partly supported by contract *AT*–04–1–*GEN*–12 between the Atomic Energy Commission and the University of California, Los Angeles; aided, in part, by grants from the Eastern Seal Foundation, Society for Crippled Children and Adults; Ayerst Research Laboratories, Inc.; Squibb Institute for Medical Research; the Josiah Macy, Jr., Foundation; and U.S. Public Health Service Grant-in-Aid No. *A*-3793 (*RI*).

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THE communication by Miss C-C. Mok and me in *Nature*[1] stated our belief that we had identified phosvitin in hen's serum for the first time. Dr. Schjeide and Dr. Urist suggest that their extensive researches on the proteins of avian yolk and serum were unknown to us. In point of fact, their observations, as reported in certain of their papers[2,3], had encouraged us to persevere in attempts to isolate phosvitin from hen's serum by chemical procedures. Our communication, like our work, deals with a relatively very limited part of the wide range covered by the researches of Dr. Schjeide and Dr. Urist, but we regret the omission of any reference to their

researches, the more so as a full publication of our own restricted study has been delayed.

In one of their papers[4], published prior to our communication, Dr. Schjeide and Dr. Urist point out that their serum X_1-phosphoprotein and their egg yolk X_1-phosphoprotein, which are ultracentrifugal entities, have a common origin and that both are phosphoproteins of high serine content but differing in sedimentation behaviour. They also point out that consideration of these results, in conjunction with those of other workers, led them to the conclusion that the phosvitin of Meecham and Olcott[5] is a relatively pure X_1-phosphoprotein. The different ultracentrifugal behaviour of serum X_1-phosphoprotein and yolk X_1-phosphoprotein, however, could indicate that the 'phosvitins' from them might differ in such properties as contents of nitrogen, phosphorus and serine. This could scarcely be established except by preparation and analysis of these phosvitins. Our reported results showed that phosvitin prepared from hen's serum by the method of Joubert and Cook[6] had the same contents of nitrogen and phosphorus, and showed similar behaviour on zone electrophoresis on paper as did phosvitin prepared from egg yolk by the same method. This, of course, does not mean that the serum X_1-phosphoprotein and yolk X_1-phosphoprotein of Dr. Schjeide and Dr. Urist are the same portein, but it does suggest that these two ultracentrifugal entities would yield similar, if not identical, phosvitins when subjected to the isolation procedure of Joubert and Cook[6].

The criticism in the foregoing communication that our phosvitin preparation is a derived protein, and in that sense an artefact, may very well be valid; and if so, then we would have been more correct to have said that we believed this to be the first occasion on which phosvitin had been prepared from hen's serum by a biochemical procedure rather than "as the first occasion on which phosvitin had been identified as a constituent of the hen's blood". For the same reasons, we consider that our communication would have been more correctly entitled "Preparation of Phosvitin from the Serum of the Hen". We also suggested[1] that two lipoprotein zones observed on paper electropherograms of egg yolk and hen's serum might be related to a lipovitellin–phosvitin–γ-livetin complex and to lipovitellenin. In the light of later work, we now regard this suggestion as invalid, at least in so far as it relates to lipovitellenin. The resolution of lipoprotein zones on such electropherograms is greatly affected by the nature of the buffer.

Certainly ultracentrifugal studies, such as those of Dr. Schjeide and Dr. Urist, are likely to separate entities much more closely related to the physiological entities present in serum than are biochemical procedures or zone electrophoresis. Indeed, it is not at all easy to relate the results of zone electrophoretic experiments to the results of free electrophoretic studies, such as those of Clegg and his collaborators (for example, refs. 7–9). At the same time, simpler biochemical procedures and zone electrophoresis are more widely available techniques than ultracentrifugation and free electrophoresis; and to that extent it may be worth while to persist with the simpler techniques and to attempt to relate the results to those established by more elaborate and fundamental approaches, such as those of Dr. Schjeide and Dr. Urist, whose communication provides a timely synopsis.

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NEW EVIDENCE FOR A RELATIONSHIP BETWEEN ION ABSORPTION AND PROTEIN TURNOVER IN PLANT CELLS

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SALT absorption in plants is clearly an energy-requiring process; but the mechanism by which it is achieved remains obscure[1]. Experiments with metabolic inhibitors such as cyanide, azide, and carbon monoxide have confirmed the dependence on respiration, and indicate the involvement of the cytochrome oxidase system. The effects of 'uncoupling agents' (for example, 2,4-dinitrophenol) on respiration and absorption strongly suggest that ion transport is more closely linked to the supply of high-energy phosphate compounds than to electron transport, but the evidence is not unequivocal[2,3]. Other methods of separating ion absorption from respiration must be exploited if their interrelationships are to be fully elucidated.

Steward and Preston[4] were impressed by the closely similar influence of various external factors on salt accumulation and protein synthesis in potato tuber slices, and a scheme has been proposed whereby the two processes may be linked[5]. Other investigators[6–8] pointed out, however, that active absorption can occur in excised roots and storage tissue cells in the absence of net protein synthesis (compare ion movements in erythrocytes, nerve and muscle cells of animals); and such claims led to the generally accepted view that the correlation sometimes observed is only an indication of a common dependence on respiration. Nevertheless, it remains possible that ion transport is linked to protein turnover (that is, breakdown and re-synthesis) which is a characteristic feature of cyto-

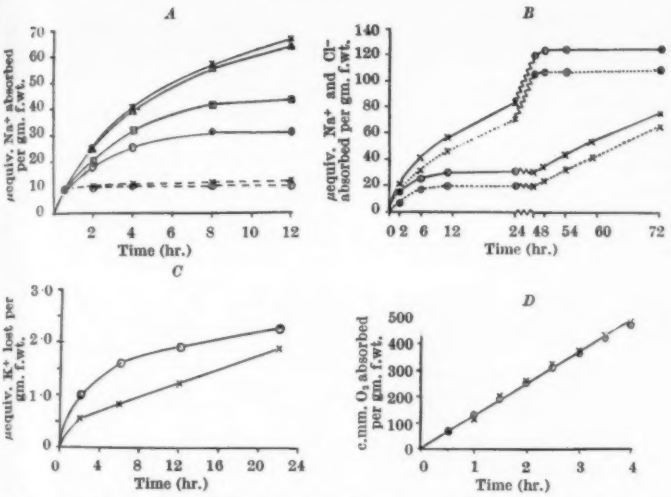

Fig. 1. Effect of chloramphenicol on: *A*, sodium absorption; *B*, sodium and chloride absorption; *C*, potassium leakage; *D*, oxygen absorption, by washed red beet slices. *A*, Sodium uptake from (*a*) 0·01 *M* sodium chloride (×); (*b*) 0·01 *M* sodium chloride + chloramphenicol (0·2 gm. per litre) (△); (*c*) 0·01 *M* sodium chloride + chloramphenicol (1 gm. per litre (□); (*d*) 0·01 *M* sodium chloride + chloramphenicol (2 gm. per litre) (○). (——) 25° C. (– – –) 2° C.

B, Sodium (——) and chloride (. . . .) uptake from 0·01 *M* sodium chloride (×) and 0·01 *M* sodium chloride + chloramphenicol (2 gm. per litre) (○) at 25° C. After 48 hr., disks were transferred into the alternative medium.

C, Potassium released from disks placed in 0·01 *M* sodium chloride (×) and 0·01 *M* sodium chloride + chloramphenicol (2 gm. per litre) (○) at 25° C.

D, Oxygen absorbed by disks placed in 0·01 *M* sodium chloride (×) and 0·01 *M* sodium chloride + chloramphenicol (2 gm. per litre) (○) at 25° C.

plasmic proteins, even under conditions which do not favour net synthesis. This possibility may be investigated experimentally using antibiotics which are known to be specific inhibitors of protein metabolism, at least in bacteria[9,10].

The effect of bactericides on uptake of salt does not seem to have been investigated hitherto with higher plants, although absorption of some of these substances has been studied[11]. Dumont[12] showed that active transport of sodium ions out of the gills of a crab, *Eriocheir sinensis*, is strongly inhibited by some antibiotics, and unaffected by others.

In the present investigation the effects of chloramphenicol on ion absorption and respiration in red beetroot and carrot root slices have been examined. The first experiment with washed beet slices showed that the antibiotic at a concentration of 1 and 2 gm. per litre (about 0·003 and 0·0006 *M* respectively) causes nearly complete inhibition of sodium absorption within about 8 hr., while at a concentration of 0·2 gm. per litre it is almost without effect (Fig. 1*A*). That the initial physical phase of cation absorption is largely unaffected by chloramphenicol was shown by the absence of an inhibitory effect during the first 2 hr. when an experiment was performed at low temperature. Likewise, the equilibration phase of rapid metabolic absorption observed with washed slices, which is completed in about 8 hr., seems to be insensitive to the inhibitor, but the prolonged slower phase of active accumulation is strongly inhibited (Fig. 1*B*). A comparable effect on chloride, as on sodium absorption, was observed. The difference between the course of absorption of the two ions is attributable to a smaller initial physical component in the case of anion than cation uptake. The concomitant slow leakage of potassium ions from cells during absorption of sodium and chloride is enhanced by chloramphenicol in the early stages of an experiment, but is reduced later (Fig. 1*C*).

The effect of chloramphenicol on sodium and chloride absorption by storage tissues is completely reversible. When red beet disks which had been kept for 48 hr. in an aerated sodium chloride solution containing chloramphenicol were washed and transferred to a solution of salt alone they began to absorb immediately, at a rate comparable to that of the control tissue (Fig. 1*B*). Conversely, absorption of salt by tissue which had been absorbing sodium and chloride for 48 hr. was almost immediately inhibited on transference to a salt solution containing chloramphenicol.

A similar experiment has been performed with washed slices of carrot tissue, and the inhibition reversed 3 times (Fig. 2*A*). A somewhat higher concentration (4 gm. per litre) is required to produce

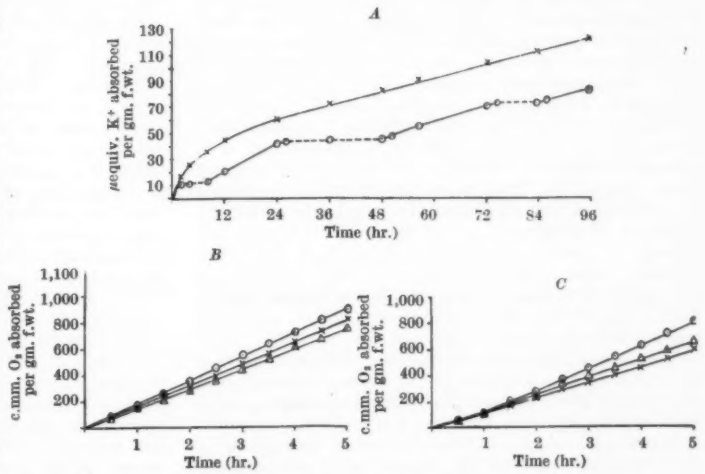

Fig. 2. Effect of chloramphenicol on: *A*, potassium absorption; *B* and *C*, oxygen absorption by washed carrot slices at 25° C.

A, Uptake from (*a*) 0·01 *M* potassium chloride and (*b*) 0·01 *M* potassium chloride + chloramphenicol (4 gm. per litre) (○– – –○) and 0·01 *M* potassium chloride (○——○) supplied alternately.

B, Oxygen absorption by disks placed in (*a*) 0·01 *M* potassium chloride (×); (*b*) 0·01 *M* potassium chloride + chloramphenicol (2 gm. per litre) (△); and (*c*) 0·01 *M* potassium chloride + chloramphenicol (4 gm. per litre) (○).

C, Oxygen absorption by disks placed in (*a*) distilled water (×); (*b*) chloramphenicol (2 gm. per litre) (△) and (*c*) chloramphenicol (4 gm. per litre) (○)

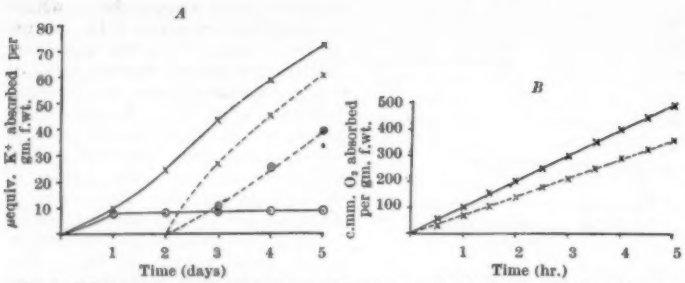

Fig. 3. Effect of chloramphenicol (2 gm. per litre) on *A*, the development of ion absorptive capacity; *B*, the enhancement of respiration, caused by washing red beet tissue at 25° C. in aerated solutions. The freshly cut tissue was washed for 8 hr. in running tap water, and afterwards for 12 hr. in distilled water, before the commencement of the experiment.

A, Uptake of potassium by disks placed in (*a*) 0·01 *M* potassium chloride immediately (×——×); (*b*) distilled water for 2 days, and then transferred to 0·01 *M* potassium chloride (×– – –×); (*c*) 0·01 *M* potassium chloride + chloramphenicol (2 gm. per litre) immediately (○——○) and (*d*) chloramphenicol (2 gm. per litre) for 2 days, and then transferred to 0·01 *M* potassium chloride (○– –○)

B, Oxygen absorption by disks placed in 0·01 *M* potassium chloride at 25° C. after (*a*) washing for 48 hr. in aerated distilled water at 25° C. (×——×); and (*b*) washing for 48 hr. at 25° C. in aerated distilled water containing chloramphenicol at a concentration of 2 gm. per litre (×– – –×)

complete inhibition of absorption in carrot than in red beet tissue.

The effects of chloramphenicol on salt absorption are manifested without any apparent influence on the rate of oxygen absorption in red beet tissue (Fig. 1*D*), and only a relatively small effect was observed in carrot (Fig. 2*B*). Calo and Varner[13] similarly noted the insensitivity of respiration, in both freshly cut and washed potato tuber slices, to inhibition by the antibiotic. In carrot, oxygen absorption is stimulated somewhat by chloramphenicol in the absence of salt (Fig. 2*C*). This is possibly attributable to release of salt from the vacuoles with a result that ions gain access to the respiratory sites in the cytoplasm. The presence of salt in the external medium after the experiment supports this contention.

The observations indicate that inhibition of ion transport induced by chloramphenicol is independent of an effect on respiration. Thus, it seems unlikely that absorption is directly related to electron transport as in the mechanisms suggested by Lundegårdh[2] and Conway[14]. Furthermore, chloramphenicol at the concentration used does not influence protoplasmic streaming in either red beet or in *Elodea* leaf cells (unpublished data), which proves that energy continues to be available at least for some endergonic processes in presence of the antibiotic. Protoplasmic streaming probably depends on a rhythmic folding and unfolding of protein molecules, and it is therefore unlikely that chloramphenicol would inhibit ion absorption if uptake occurred by a mechanism dependent solely on this phenomenon, such as that suggested by Goldacre and Lorch[15].

If it is assumed that chloramphenicol prevents salt absorption by inhibiting the same reaction as that affected in blocking protein metabolism, then a close relationship between the two processes is indicated. Supporting evidence for this conclusion is supplied by the inhibitory effects of nucleic acid antagonists, ribonuclease, and ultra-violet light on both ion absorption[16,17] and on protein synthesis[9,10]. It must be noted, however, that the concentration of chloramphenicol necessary to inhibit ion transport in carrot root slices is at least 10 times greater than that required to prevent growth of carrot callus tissue cultures under the influence of coconut milk (unpublished work). The reason for this discrepancy is still obscure; but differences in the rate of penetration of the antibiotic into the two kinds of tissue may be responsible.

The manner in which ion transport could be linked to protein turnover or synthesis remains to be considered. It must first be emphasized that turnover probably does not involve breakdown of a complete protein molecule followed by immediate re-synthesis, but rather the continuous removal and replacement of individual amino-acids in the polypeptide chain[18]. A similar series of reactions to those involved in the exchange process is probably concerned also in synthesis of new protein, and, in fact, in *in vitro* experiments on incorporation of labelled amino-acids it is difficult to distinguish between exchange and net synthesis. No attempt to do this is made in the discussion which follows. Both processes involve temporary attachment of the protein molecule, or a part of it, to a ribonucleic acid template, and it seems likely that the formation of such a protein-nucleic acid complex diminishes the capacity of the protein to bind ions both by removing available electrostatic binding sites and by reducing its chelating power, thus causing ions to be released. When the turnover site leaves the template and returns to its natural shape, binding capacity is perhaps restored and ions are taken up again. Although probable, this idea is hypothetical since no direct evidence for the reversible release of ions from protein is yet available. The ability of proteins to bind ions with a specificity comparable to that exhibited by ion transport mechanisms is well illustrated by the association of enzymes with specific activating ions.

In order that a molecule may function as an ion carrier it must not only possess the ability to bind ions reversibly, but it must also be suitably located in a membrane. The association of proteins with both surface and internal cytoplasmic membranes is quite certain, but the manner in which they are arranged in relation to the lipid and phospholipid components remains uncertain. It is likely that the membranes are in a state of continuous flux, and as a result of such movements a particular region of a protein molecule may occasionally face towards one surface of a membrane, and then the other, especially when the membrane is thin. Thus, ions which are picked up at a surface in contact with the medium may be released again on the inside where the amino-acid exchange mechanism is located. The proximity of the ribonucleic acid template may prevent the carrier site from binding ions on the inner surface immediately after exchange has occurred, and allow it to traverse the membrane unloaded before accepting ions again from the medium. The characteristic location of microsomes, which are known to be particularly active in facilitating protein turnover and synthesis, on one side of the membranes of the endoplasmic reticulum[19] may be of significance in determining the direction of ion transport across cytoplasm. However, no such arrangement occurs at the vacuolar boundary of a plant cell, and yet ions accumulate in the central vacuole. Moreover, the probable thickness of the tonoplast is such that the mere turn-about of indivi-

dual protein-binding sites is unlikely to facilitate transport across the membrane as a whole. Either the ions are handed from site to site in moving from the outer to the inner surface, or other substances, for example, phospholipids, collaborate in a manner which is yet obscure. Possibly, ions are accumulated first in some of the smaller vacuoles and vesicles with which the cytoplasm abounds, and are then transferred to the central vacuole as a result of coalescence between it and the smaller ones. Vacuole coalescence is a feature of vacuome development in meristematic cells, and may well persist even in mature parenchyma.

Possibly only a few types of proteins are suitably adapted to carry out ion transport in the manner proposed. It has been suggested[20,21], that specific proteins with enzyme-like properties (permeases and translocases) are concerned in the transfer of sugars and phosphate across bacterial membranes. Chloramphenicol may be a specific inhibitor of such carriers functioning in the plant cell, and this possibility needs to be investigated further. It is interesting to speculate that, alternatively, the capacity to bind ions reversibly may be an inherent property of all labile protein molecules, and those which are located in membranes can function as carriers in active transport. All membranous organelles may thus be capable of ion accumulation, such as has been shown in the case of mitochondria[22,23]. Inhibition by chloramphenicol is then attributable to a general effect on protein turnover.

A feature of cells of storage tissues is that when first cut from the dormant organ they are incapable of accumulating salt, but a capacity to do so develops if they are washed for a time in an aerated solution of salt or in aerated distilled water. The development of absorptive ability has been attributed to synthesis or activation of carrier molecules resulting from enhanced metabolism induced by this treatment[8]. An experiment in which disks of red beet were washed either in a salt solution or in distilled water, in the presence and absence of chloramphenicol, has shown that the antibiotic prevents the development of absorptive capacity. Disks washed for 2 days in chloramphenicol (2 gm. per litre) at 25° C., and then transferred to salt, absorbed potassium over the next 3 days at an increasing rate comparable to that of disks placed in salt solution at the start (Fig. 3*A*). Disks washed for 2 days in chloramphenicol and transferred to salt also showed a lower rate of respiration than disks washed for the same time in distilled water before transference (Fig. 3*B*). A similar observation was made by Calo and Varner[13] with potato tuber tissue. The antibiotic evidently inhibits some metabolic activity upon which stimulation of both respiration and ion accumulation capacity depend.

My thanks are due to Miss E. R. Counter and Mr. A. G. Smithers for assistance in carrying out the experiments described. I am grateful to Dr. R. M. Klein of the New York Botanical Garden, who suggested to me that a study of the effect of a specific inhibitor of protein synthesis on ion absorption might prove interesting; and to Dr. J. Chayen of the Pathology Department, Royal College of Surgeons of England, for an exciting discussion of the implication of the results obtained.

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PHOSPHOLIPIDS CONTAINING PHOSPHATE TRIESTER GROUPS

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THE study of phospholipids has undergone rapid development in recent years not only in respect of the number of papers published annually but also because findings in the fields of respiratory enzymes[1,2], blood coagulation[1], cell structure[1] and transport across cell membranes[1,3] emphasize the importance of phospholipids. Much of this activity has been stimulated by the introduction of new analytical procedures which have greatly simplified the techniques used in this field. Dawson[4] showed that mild alkaline hydrolysis (0·2 *N* sodium hydroxide at 37° for 20 min.) of phospholipids yielded a mixture of glyceryl phosphoryl choline, glyceryl phosphoryl ethanolamine, glyceryl phosphoryl serine, glyceryl phosphoryl glycerol, glyceryl phosphoryl inositol and glycerol-1-phosphoric acid which could be separated by paper chromatography. It was assumed that each water-soluble phosphate ester was derived from one phospholipid and hence the specific radioactivity of each phospholipid, if labelled with phosphorus-32, could be deduced from that of the corresponding water-soluble phosphate ester. Chromatography on silicic acid was shown by Lea, Rhodes and Stoll[5] to effect a separation of phospholipids into well-defined components, and Marinetti, Erbland and Stotz[6] obtained good separations using chromatography on silica-impregnated paper and di-*iso*butylketone/acetic acid/water (40:25:5 by volume).

Table 1. Rat Liver Phospholipids

All results expressed as moles per cent of total lipid-phosphorus

	Hanahan *et al.* (ref. 8)	Shotlander and Collins (ref. 9)
Phosphatidyl ethanolamine and serine	22	18
'Complex' phospholipids	—	39
Lecithin	58	33
Lysolecithin	3	—
Minor components*	13	10
	96	100

* Phosphatidyl inositol, sphingomyelin and phosphatidic acid-like materials.

It was surprising to find that the analytical methods[7] introduced in this laboratory gave results that were at variance with those obtained by the procedures referred to above. In this laboratory the phospholipids are dinitrophenylated using 1-fluoro-2:4-dinitrophenyl-benzene followed by methylation using diazomethane, and the resulting phospholipid derivatives are separated, by low-temperature chromatography on cellulose at −20° using acetone, into fractions soluble and insoluble respectively in acetone, and then each fraction is further resolved by counter-current distribution. Table 1 shows a comparison with the results obtained by Hanahan, Dittmer and Warashina[8], who used silicic acid chromatography. The major difference is in the 'complex' phospholipids which constitute 39 per cent of the total rat-liver lipids and in which the molecular weight is at least double that of the known phospholipids. This fraction has not been detected by other workers.

There are two possible explanations for this discrepancy: one is that the 'complex' phospholipids are degraded by the silicic acid chromatography, and the other is that the chemical treatment (dinitrophenylation and methylation) used in this laboratory[7] results in the production of artefacts not existing in the original phospholipid mixture.

To test these alternatives, rat liver lipids were treated with silicic acid (Mallinckrodt), the lipids recovered quantitatively, dinitrophenylated and methylated and separated by counter-current distribution into two fractions: *A*, that containing phosphatidyl ethanolamine and phosphatidyl serine; *B*, that containing the 'complex' phospholipids. The amounts of these compounds were determined from the content of dinitrophenyl-groups and the results are shown in Table 2. Not only was the proportion in fraction *B* of the 'complex' phospholipids decreased, but also further examination revealed considerable qualitative changes both in respect of their behaviour on counter-current distribution and in their solubility in acetone and in water. The dinitrophenylated and methylated 'complex' phospholipids are insoluble in water and very soluble in acetone but, after treatment with silicic acid, only 6–10 per cent of the total phospholipids had these properties. Of the remaining dinitrophenyl-containing materials in fraction *B*, 4 per cent (of the total phospholipids) were insoluble in acetone and 3 per cent were soluble in water. The small amount of 'complex' phospholipid which appeared to be unchanged had the molar ratio dinitrophenyl-groups/phosphorus = 0·5 but, unlike the original material, choline was not detected. The fall in the amount of 'complex' fraction was not accompanied by a corresponding rise in the phosphatidyl ethanolamine and serine, and thus there was a disappearance of about a third of the total amino-nitrogen (ethanolamine and serine). These results show that silicic acid can degrade phospholipids and that its effect is on the 'complex' fraction. Other workers[8] did not consider the possible presence of unidentified lipids which might be sensitive to silicic acid, but were content to show that silicic acid chromatography was without effect on lecithin and phosphatidyl ethanolamine.

Table 2. Effect of Silicic Acid on Phospholipids

Phospholipids from the same sample of rat liver lipids were treated with silicic acid as shown below, the lipids were then completely eluted and treated as described by Collins and Wheeldon (ref. 7).

All results are expressed as gm. atoms of phosphorus per 100 gm. atoms of total lipid phosphorus.

Fraction	*A* Phosphatidyl ethanolamine and serine	*B* 'Complex' phospholipids
Treatment		
Control	15	32
Lipids (300 mgm.) stood with 12 gm. silicic acid in chloroform-methanol (1:1 by vol.) for 48 hr.	15	20
Lipids (300 mgm.) chromatographed on silicic acid (12 gm.)-'Celite' (6 gm.) Rate of flow 3–4 ml./min. Solvents, chloroform-methanol in the proportions used by Hanahan *et al.* (ref. 8)	11	18

While the above evidence demonstrates that silicic acid can degrade some phospholipids, this of itself does not dispose of the possibility that the process of dinitrophenylation and methylation may result in artefacts. Reasons for believing otherwise are discussed in previous papers[7], and are augmented by the following experiment (Table 3) in which the object was to show that the specific radioactivity of the phosphorus, esterified to ethanolamine, in phosphatidyl ethanolamine is greater than that in the complex phospholipids. As the methods available did not separate the serine component, the specific radioactivity of the ethanolamine component was estimated from the specific radioactivity of the combined fraction on the assumption that the radioactivity of the serine component was zero. Although this is not the case[10,11], it is the least favourable assumption that can be made in that it minimizes the differences between the two ethanolamine-containing materials. The phosphatidyl ethanolamine and serine (as the dinitrophenylated and methylated derivatives) were isolated by counter-current distribution and redistributed in heptanemethanol for 120 transfers. A single dinitrophenyl-containing peak was obtained with a partition coefficient of 0·50, in which the molar ratio of dinitrophenyl-groups/phosphorus was 1·0 and which contained 86 per cent phosphatidyl ethanolamine and 14 per cent phosphatidyl serine. The specific radioactivity of this material is shown in Table 3. Because the 'complex' phospholipids contain approximately two atoms of phosphorus to each molecule of ethanolamine or serine, it was necessary to effect a partial hydrolysis before the radioactivity could be related to the amino-compounds. The complex fraction was treated with 0·2 *N* sodium hydroxide in 50 per cent aqueous methanol for 20 min. at 37°, the mixture neutralized with 'Zeokarb-225' (H-form), and the fatty acids removed by extraction with diethyl ether. Paper chromatography in phenol-water of the water-soluble materials showed the presence of two phosphate-containing[12] spots (R_F 0·73 and 0·54). Both spots gave a positive reaction for a *cis*-glycol group[13]; the slower spot has not yet been identified, but the faster-moving material was yellow, indicating

Table 3. INCORPORATION OF RADIOPHOSPHORUS INTO PHOSPHATIDYL ETHANOLAMINE AND THE ETHANOLAMINE-CONTAINING PORTION OF THE 'COMPLEX' PHOSPHOLIPIDS

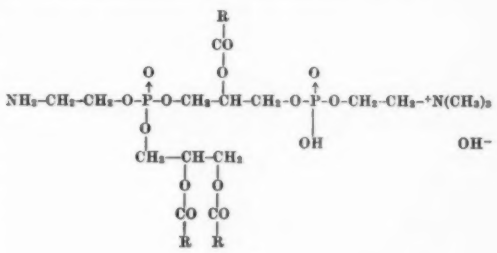
A rat was intravenously injected with 0·8 mc. of sodium dihydrogen phosphate-^{32}P (0·01*M*, *p*H 7·0) in 0·1*M* sodium citrate (*p*H 7·0), killed 90 min. later, the liver lipids extracted, and processed as described by Collins and Wheeldon (ref. 7) and Shotlander and Collins (ref. 9).

	Specific radioactivity (counts/min./μmole P)	Ratio serine-nitrogen to total amino-nitrogen	Specific radioactivity of ethanolamine moiety, assuming that of serine is zero (counts/min./μmole P)
Phosphatidyl ethanolamine and serine	13,700	0·14	15,900
'Complex' phospholipids (phosphorus esterified directly with ethanolamine and serine)	9,100	0·24	12,000

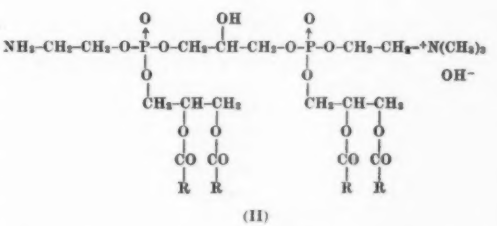
the presence of a dinitrophenyl group. The yellow spot was eluted with water, and analysis indicated that the molar ratio of dinitrophenyl-groups/phosphorus was 1·0, and it was concluded that this was glyceryl phosphoryl N-2:4-dinitrophenyl ethanolamine (or serine, as no separation was obtained in this solvent system). The specific radioactivity and serine content of this material are shown in Table 3. It will be seen that the specific radioactivity of the phosphatidyl ethanolamine is between 1·50 and 1·32 times that of the phosphate ester containing ethanolamine, which was derived from the complex fraction and, as the combined counting and chemical errors do not exceed 4 per cent, this difference is highly significant.

These results show that the dinitrophenylated and methylated 'complex' phospholipids represent a distinct and real fraction, but beyond indicating that they are sensitive to silicic acid, they provide no information as to their structure. Previous work[7] has shown that the molar ratio of dinitrophenyl-groups/phosphorus is usually 0·5 but may be 0·67. Qualitative analysis[7,9] has also shown that choline is present in some but not all the components that make up the 'complex' phospholipid fraction and that inositol is absent. Further information was obtained in experiments in which the dinitrophenylated and methylated 'complex' phospholipid fraction (50 mgm.) was treated with one of the following reagents: triethylamine (0·1 ml. in 10 ml. of diethyl ether saturated with water and left 12 hr. at 20°), tetramethyl ammonium hydroxide (0·1 ml. of a 25 per cent (w/v) aqueous solution added to 10 ml. of diethyl ether saturated with water and left 12 hr. at 20°), acetic acid (8 ml. of glacial acetic acid and 2 ml. water and left 30 min. at 40°). In addition, chromatography on 'Hyflo-Supercel' or on silicic acid (Lights or Mallinckrodt) gave similar results. The total lipids were recovered after treatment, treated with diazomethane and submitted to counter-current distribution. One of the products isolated had a molar ratio of dinitrophenyl-groups/phosphorus of 1·0 and was indistinguishable from the methyl ester of dinitrophenyl phosphatidyl ethanolamine and serine. This methyl ester was not obtained if the preliminary 'hydrolytic' treatment was omitted, and hence one must conclude that the preliminary treatment gave rise to dinitrophenyl phosphatidyl ethanolamine or serine. The only groups present in these compounds which are available for chemical bonding are the free hydroxy group on the phosphorus and the carboxy group on the serine, and it thus follows that the 'complex' phospholipids contain either a phosphate triester or a serine-carboxylic ester. This last alternative is unlikely, not only because it could account for only 24 per cent of the 'complex' phospholipids but also because carboxylic esters such as the glycerides appear to be quite stable to silicic acid chromatography. Thus, one must infer that the 'complex' phospholipids are distinguished from the remaining phospholipids, which are phosphate diesters, by the presence of a tertiary ester of phosphoric acid. The nature of the compound to which the phosphatidyl ethanolamine is esterified has not been fully elucidated, but it is clear that it must contain phosphorus and is in all probability another phospholipid.

Two types of compounds are possible: (1) those in which phosphatidyl ethanolamine is esterified with the free hydroxy group in such a compound as lysolecithin, sphingomyelin or cardiolipin; (2) the second type can be imagined in which phosphatidyl ethanolamine is linked by glycerol (or similar compound) to another phospholipid and the resulting compound would have two phosphate triester groups. The suggested structures are shown below: the first type would be a phosphatidyl ethanolamine-lysolecithin, (I), whereas the second would be, for example, 1-(phosphatidyl ethanolamine) 3-(phosphatidyl choline) glycerol (II). Complete or partial hydrolysis of compounds such as those suggested above would yield products which would be identical with those which are in fact obtained, and this helps to explain their non-recognition in the past. Further, the splitting of the phosphate triester on silicic acid would either yield the classical forms (phosphatidyl choline, ethanolamine or serine) or such compounds as lysolecithin or lysophosphatidyl ethanolamine.



(I)



(II)

The occurrence of lysolecithin in animal tissues has only been reported in recent years, and it is interesting to note that in every case it was isolated either by silicic acid chromatography[5], or by chromatography on paper impregnated with silicic acid using aqueous acetic acid in the solvent system[6], or by counter-current distribution using aqueous acetic acid as a component of the solvent mixture[14]. Both silicic acid and acetic acid have been shown here to be effective

in degrading the 'complex' phospholipid fraction, and lysolecithin has not been detected by the methods in use in this laboratory. The 'complex' or triester fraction has been shown to be present in rat tissues (liver, kidney, heart, lung, brain, spleen and intestine), sheep brain, human blood plasma, influenza virus, yeast and cabbage leaf, and the amounts have varied from 15 per cent for human blood plasma to 60 per cent for sheep brain.

The fact that phospholipids consist of about 60 per cent in the form of phosphate diesters and 40 per cent as triesters means that most investigations on phospholipids have neglected the study of a major portion. The procedure described by Dawson[4] involving alkaline hydrolysis, which has been widely used for the study of the exchange of inorganic phosphorus-32 with phospholipids, cannot distinguish between the diesters and the triesters, as the triesters of phosphoric acid are labile to alkali and a diester would be formed.

The procedure[7] developed in this laboratory suffers from the disadvantage that derivatives of the phospholipids are isolated and not the native materials. In certain fields such as in the study of blood coagulation, this is a serious drawback as the object is the isolation of material with biological activity. Apart from the fact that the native 'triester' phospholipids are more soluble in ethanol than phosphatidyl ethanolamine or serine[7], no work has been attempted on their isolation in an unaltered condition. An analytical method[7] exists for the determination of this group of phospholipids and hence attempts at their isolation can be readily followed. However, the solution to the problem of obtaining the native materials may be as readily solved by chemical synthesis, and in the meanwhile, much work remains to be done on the elucidation of the structures of the phosphate triester phospholipids.

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ACTION OF SPERMINE ON SOLUBLE RIBONUCLEIC ACID AND SOME POLYNUCLEOTIDES

Spermine Fractionation of Soluble Ribonucleic Acid of Rabbit Liver

THE unique biological role of cytoplasmic, or soluble, ribonucleic acid, as an acceptor for acyl-activated amino-acids, and as a donor of the amino-acyl moiety to microsomal protein, has been established[1–3]. Furthermore, it has been found that in any one soluble ribonucleic acid preparation there are a finite number of acceptor sites, presumably one for each polynucleotide chain, and it has been shown by several investigators[2,4,5] that each amino-acid is bound to soluble ribonucleic acid in a fixed amount and independently from other amino-acids. From these observations it has been inferred that there is a specific soluble ribonucleic acid chain for each amino-acid, and indeed it has been possible to separate, at least partially, different species of soluble ribonucleic acid by various experimental procedures. These procedures[6–8] may be based on either (*a*) special properties conferred by the presence of an amino-acid at the nucleoside end, or (*b*) differences in the physico-chemical properties of the various chains. Thus Brown *et al.*[6] were able to prepare in essentially pure form the soluble ribonucleic acid species specific for tyrosine or histidine by taking advantage of the ability of soluble ribonucleic acid labelled with these amino-acids to form diazonium compounds with a polyaminoazostyrene resin at neutral *p*H. On the other hand, partial separation of amino-acid specific soluble ribonucleic acids based on physicochemical properties of the amino-acid-free soluble ribonucleic acid were obtained by Smith *et al.*[7] by column chromatography and by Bowman[8] by electrophoresis on starch. More promising results were obtained by Holley *et al.*[9] through application to soluble ribonucleic acid of the counter current distribution system of Warner and Vaimberg[10].

Purification of soluble ribonucleic acids specific for valine or proline was achieved, as described here, by utilizing the well-known ability of polyamines to form insoluble complexes with polynucleotides[11]. The procedure was based on the assumption that amino-acid-specific soluble ribonucleic acids differ sufficiently in their chemical composition (base composition or sequence) to allow separation. This assumption is in accord with the results of Holley *et al.*[9] and Warner and Vaimberg[10] and is supported directly by the findings of Klee and Cantoni[12] that the specificity of soluble ribonucleic acid for proline, valine or leucine is not related to molecular weight.

Rabbit liver soluble ribonucleic acid was isolated from the '*p*H 5 enzyme' by a sodium chloride extraction procedure to be described in a forthcoming publication. On treatment of soluble ribonucleic acid with a two- to three-fold excess of spermine tetrahydrochloride at *p*H 5·6 and low ionic strength (0·02 *M* sodium succinate buffer in 0·03 *M* sodium chloride) approximately 70 per cent of the ultraviolet absorbing material is precipitated. The sper-

Table 1. SPERMINE FRACTIONATION OF SOLUBLE RIBONUCLEIC ACID

	Amino-acid acceptor units		Specific activity (μmoles of amino-acid/125 nucleotides)		
	Proline	Valine	Proline	Valine	P/V
Rabbit liver Soluble ribonucleic acid	100	100	0·115	0·041	2·8
Supernatant	24·8	69	0·092	0·091	1·0
Precipitate:					
Extract 1	10·5	10	0·194	0·066	2·9
Extract 2	26	5	0·276	0·039	7·1
Extract 3	25·7	10	0·316	0·035	9·1

The starting material, and the various fractions were tested as follows for their ability to accept activated amino-acids: the reaction mixtures (0·5 ml.) contained the following components; adenosine triphosphate, 10 m*M*; magnesium chloride, 20 m*M*; sodium chloride, 2·8 m*M*; potassium phosphate buffer, *p*H 7·6, 2·8 m*M*; ^{14}C amino-acid, 0·05 m*M*; and approximately 8 optical density units of soluble ribonucleic acid. Appropriate activating enzymes[14] were added to start the reaction; incubation time, 15 min. at 37°. The reaction was stopped by addition of 0·5 ml. of 6 per cent chloroform. The precipitate was washed 3 times with 3 per cent chloroform, once with ethanol, suspended in 0·75 ml. of 6 per cent chloroform, and hydrolysed for 30 min. at 100°. After neutralization with potassium hydroxide, the optical density of this solution was determined and an aliquot was plated and counted. Values are corrected for self absorption. Amino-acid acceptor units thus determined are in counts/min./o.d. at 260 mμ.

mine-soluble fraction is 2–3 times richer in valine-specific soluble ribonucleic acid than the starting material. Repeated extraction of the spermine-insoluble fraction with small volumes of phosphate buffer, 0·025 *M*, *p*H 7·2 in 0·05 *M* sodium chloride, yields a fraction enriched by about the same factor with respect to proline acceptor ability. Total recovery in both cases is satisfactory (Table 1). On the assumption that in a single soluble ribonucleic acid chain there is only one acceptor site for the activated amino-acid and on the basis of a chain-length of 125 nucleotides, the partially purified proline specific soluble ribonucleic acid is approximately 25–30 per cent pure, while the valine specific species is about 8–10 per cent pure. (The value of 125 for chain-length is based on a molecular weight of 45,000–55,000, as determined by sedimentation and viscosity measurements[13].)

It is noteworthy that the *p*H at which the fractionation is carried out is relatively critical. Thus, essentially no fractionation was achieved at *p*H 6 or 4·8. At *p*H 5·2 the results obtained are in the same general direction but partition is less precise. Intermediate *p*H's have not yet been investigated. Since the ability of spermine to react stoichiometrically with soluble ribonucleic acid is not dependent on *p*H, these results suggest that the determining feature which permits fractionation of a population of various soluble ribonucleic acid chains relates to the pK_a of the amino group of cytosine (and/or adenosine) in the polynucleotides.

Work by Huang and Felsenfeld using synthetic polynucleotides as model systems provides support for this hypothesis. These results are detailed in the following communication. It is of interest to point out that rabbit liver soluble ribonucleic acid has a high cytosine content[15], as is also the case for soluble ribonucleic acid derived from *E. coli* or yeast[16,17].

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Solubility of Complexes of Polynucleotides with Spermine

Cantoni has shown in the preceding communication that the solubility of soluble ribonucleic acid-spermine complexes is dependent on *p*H. The solubility of nucleic acid complexes with poly-cations has also been the subject of investigations by Razin and Rozansky[1], who studied polyamine-nucleic acid interactions, and by Chargaff *et al.*[2], who used polylysine and other poly-cations to fractionate deoxyribonucleic acids. The results presented in this communication are concerned with the behaviour of complexes of spermine with synthetic polyribonucleotides.

If an equivalent of spermine per mole of polymer phosphorus is added to a distilled water solution of heat-denatured calf thymus deoxyribonucleic acid at *p*H 6·5, a precipitate forms at once and most of the nucleic acid may be separated by centrifugation. Similar results were obtained with the synthetic polyribonucleotides, polyadenylic acid and polyinosinic acid. The polypyrimidines, polycytidylic acid and polyuridylic acid formed somewhat more soluble complexes with spermine, though precipitation was observed in the presence of excess spermine. Under conditions similar to those of some of the experiments described here (polymer phosphate concentration about 5×10^{-5} *M*, buffer concentration about 0·01 *M*, spermine concentration about 2×10^{-4} *M*), addition of spermine to separate solutions of polyadenylic acid, polyinosinic acid, polycytidylic acid and polyuridylic acid resulted in immediate extreme elevation of the absorption spectrum because of scattering from aggregates. Centrifugation at 16,000 *g* in a Servall centrifuge for 10 min. was sufficient to remove from suspension 80 per cent or more of the polyadenylic, polyinosinic and polycytidylic acids. Only 50 per cent of the polyuridylic acid-spermine was removed, but the spectrum of the supernate showed that the remaining material was in an aggregated form. The same solubility properties were observed for all these polynucleotides at *p*H 6·8 (cacodylate buffer) and *p*H 4·1 (acetate buffer).

When polyadenylic acid ($S_{20} = 8{\cdot}4$) and polyuridylic acid ($S_{20} = 5{\cdot}4$) are mixed in 1:1 base ratio in the presence of 2×10^{-3} *M* magnesium chloride, the two-stranded poly (adenylic + uridylic) complex is formed[3]. (Sedimentation coefficients, given in Svedberg units, were measured in 0·05 *M* sodium chloride, 0·005 *M* cacodylate buffer, *p*H 6·8, using

a Spinco model *E* ultracentrifuge equipped with ultra-violet optical system. According to Fresco and Doty[8] the value of S_{20} found for polyadenylic acid corresponds to a molecular weight of about 4×10^5.) The addition of an equivalent of spermine to this complex at *p*H 6·8 (or any other *p*H between 6·8 and 4·5) caused total precipitation. However, if polyinosinic acid ($S_{20} = 6·3$) and polycytidylic acid ($S_{20} = 5·2$) were similarly caused to combine in 1 : 1 ratio[4] by adding magnesium chloride, the complex formed when spermine was added was soluble at *p*H 7. (All the observations concerning solubility of spermine complexes were made within 2 hr. after addition of spermine. After 24 hr. small amounts of precipitation have been observed in poly (inosinic + cytidylic)-spermine at *p*H 6·8.)

The solubility of the poly (inosinic + cytidylic)-spermine complex has been studied as a function of *p*H. To a 1-ml. sample of 5×10^{-5} *M* poly (inosinic + cytidylic) in 2×10^{-3} *M* magnesium chloride was added 0·1 ml. of 0·1 *M* cacodylate or acetate buffer of the appropriate *p*H. The absorption spectrum was determined in a Cary model 14 spectrophotometer, and 0·04 ml. of $1·25 \times 10^{-3}$ *M* spermine tetrahydrochloride was added. The spectrum was plotted again both before and after centrifugation at 16,000 *g* in a Servall centrifuge for 15 min. At every *p*H between 6·8 and 5·1 there was no change in the absorption spectrum (aside from dilution effects) before centrifugation, and no more than a 5 per cent decrease in optical density after centrifugation. There was no further decrease in optical density with longer periods of centrifugation. However, when *p*H 4·5 buffer was used the addition of spermine resulted in immediate aggregation and increase of absorption values throughout the spectrum. Centrifugation removed these aggregates; only 70 per cent of the original polynucleotide remained in solution. When the experiment was repeated with *p*H 4·1 buffer only 30 per cent of the poly (inosinic + cytidylic) was still soluble after centrifugation.

This behaviour is in contrast with that of poly (adenylic + uridylic) in 2×10^{-3} *M* magnesium chloride. Using conditions similar to those described for poly (inosinic + cytidylic) it was found that poly (adenylic + uridylic) precipitated completely both at *p*H 6·8 and *p*H 5·1. In the experiment shown in Fig. 1 equal volumes of 5×10^{-5} *M* poly (adenylic + uridylic) and 5×10^{-5} *M* poly (inosinic + cytidylic) (both in 2×10^{-3} *M* magnesium chloride) were mixed and 0·1 ml. of 0·1 *M* cacodylate buffer (*p*H 6·8) added to a 1·0-ml. aliquot. The resultant spectrum (curve *A*) was the mean of the spectra of poly (adenylic + uridylic) and poly (inosinic + cytidylic) taken separately. Spermine (0·15 ml. of $1·25 \times 10^{-3}$ *M*) was added directly to the cuvette. The spectrum gave evidence of aggregation and after centrifugation only the spectrum of the poly (inosinic + cytidylic) remained, as demonstrated by direct comparison with a poly (inosinic + cytidylic) control containing the same concentrations of poly (inosinic + cytidylic) and of spermine used in the solution of curve *A*. Spermine thus effects a complete separation of poly (inosinic + cytidylic) from poly (adenylic + uridylic) under these conditions.

It does not appear likely that the differences in solubility described here were the result of differences in molecular weight. The polyinosinic and polycytidylic acids used in these experiments were precipitable, and so was incompletely combined poly (inosinic + cytidylic), or completely combined polymer at *p*H 4·1. With smaller polyinosinic and polycytidylic acids (S_{20} equal to 2·6 and 2·1 respectively) the addition of polycytidylic acid-spermine solution to the polyinosinic acid-spermine precipitate caused rapid solution of the polyinosinic acid and immediate appearance of the poly (inosinic + cytidylic) spectrum. The simplest conclusion is that only the well-ordered poly (inosinic + cytidylic) structure[4] is capable of forming soluble complexes with spermine. The precipitation of the poly (inosinic + cytidylic)-spermine complex formed at low *p*H may be the result of partial derangement of the poly (inosinic + cytidylic) structure as cytosine is titrated[5] in the neighbourhood of *p*H 4·5. The spectral shifts that occur when the *p*H is lowered to 4·5 indicate that cytosine is being titrated, though this does not necessarily mean that the hydrogen bonding to inosine has been destroyed[6]. If structure is important, then it must be assumed that the poly (adenylic + uridylic) structure[7] differs from that of poly (inosinic + cytidylic) sufficiently to affect the solubility properties.

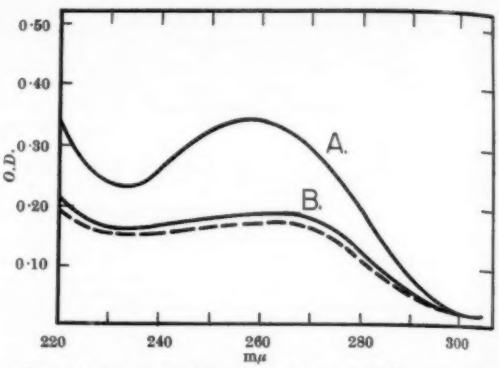

Fig. 1. Absorption spectrum of a solution originally containing $2·3 \times 10^{-5}$ *M* poly (adenylic + uridylic), $2·3 \times 10^{-5}$ *M* poly (inosinic + cytidylic), and 0·091 *M* *p*H 6·8 cacodylate buffer. Total volume 1·1 ml. Curve *A*, before addition of spermine; Curve *B*, solid line, after adding 0·15 ml. of $1·25 \times 10^{-3}$ *M* spermine and centrifuging. Dotted line, a solution containing $2·0 \times 10^{-5}$ *M* poly (inosinic + cytidylic, 0·080 *M* cacodylate buffer, and $1·5 \times 10^{-4}$ *M* spermine. In all experiments spermine was added as the tetrahydrochloride

The similarity of the behaviour of these synthetic polymers to that of soluble ribonucleic acid may be accidental, but there is little doubt that fractionation of ordered polynucleotides may be effected with spermine, and that this fractionation has a *p*H dependence resembling that of soluble ribonucleic acid. Further investigations with complexes of the co-polymers poly (adenylic; uridylic) and poly (inosinic; cytidylic) are in progress.

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LETTERS TO THE EDITORS

PHYSICS

Standard Unit of Pressure in Vacuum Physics

A UNIT of pressure in terms of the height of the column of a liquid manometer is essential, since such an instrument, including the compression manometer (McLeod gauge), is indispensable in the measurement of low pressures. Even in the recent methods[1] developed for measuring pump speeds and calibrating vacuum gauges, in which the only magnitude needed to be determined accurately is a rate of gas flow, manometers are required.

The use of the existing practical unit (mm. mercury) has already been challenged by the tremendous development of the physics and technology of vacuum. This unit is characterized by the following features: (1) It does not express a pressure in the basic terms of force per unit area. (2) It is not related decimally to the absolute units of pressure. (3) Its value is affected by temperature and geographical position. While the third characteristic appears to be a disadvantage which can be eliminated by the application of some corrections, the second characteristic would require fundamental alteration in the definition of the unit.

The pressure h in mm. mercury is evaluated by applying the hydrostatic equation $p = g\,\Delta\,uh$, where p is the absolute pressure in C.G.S. units and $u = 1$ mm. $= 0{\cdot}1$ cm. is the unit of the column height. Since gravity $g = 980{\cdot}665$ cm. s.$^{-2}$ and mercury density $\Delta = 13{\cdot}5951$ gm. cm.$^{-3}$ at 0° C. are considered as standard values, we obtain for $h = 1$ mm. mercury:

$$1 \text{ mm. mercury} = g\,\Delta\,u = 1{\cdot}333,22 \times 10^3 \text{ dyn. cm.}^{-2}$$

All previous suggestions[2] aimed at standardization of the pressure unit have not sought to alter this relationship in such a way that p and h would become simply related. In the following I will discuss this possibility as a practical proposition.

The main point is to transform the above relation into:

$$g\,\Delta\,u = 10^n$$

where n is an arbitrary integer. Here only u and g can vary, the latter factor being limited between 978 and 983, the gravity at the equator and pole, respectively. It results that u has two limits $u_1 = 0{\cdot}07482 \times 10^{n-3}$ cm. and $u_2 = 0{\cdot}07526 \times 10^{n-3}$ cm. An acceptable magnitude for practice would be $u' = 0{\cdot}075$ cm. $= 3/4$ mm. From this assumption it follows $n = 3$ and for gravity the value $g' = 10^3/75\,\Delta$ cm. s.$^{-2}$ or $g' = 980{\cdot}7455$ cm. s.$^{-2}$ within one part in 10^5.

Thus we have:

$$p = g'\,\Delta\,u'h' = 10^3h' \text{ dyne cm.}^{-2}$$

The pressure corresponding to $h' = 1$, that is, $p = 10^3$ dyne cm.$^{-2}$, may be taken as standard unit and conveniently termed 'vac'. This pressure unit, 1 vac $= 10^3$ dyne cm.$^{-2}$, corresponds to a mercury column of exactly $0{\cdot}75$ mm. height under the conventional conditions: g' as above and $\Delta = 13{\cdot}5951$ gm. cm.$^{-3}$ at 0° C. Therefore, pressures expressed in vac are measured by taking $0{\cdot}75$ mm. instead of 1 mm. as unit of the mercury column. Since $0{\cdot}7500615$ mm. mercury $= 10^3$ dyne cm.$^{-2}$, 1 vac may for all practical purposes be equated to 3/4 mm. mercury within less than one part in 10^4.

Recommendation of vac as standard unit of pressure would require the adoption of the conventional value g', which is different from the standard gravity. Such a decision will not cause any confusion because the latter value has little use in vacuum physics. However, vacuum physicists will greatly benefit by simplification of conversion to the absolute unit. No complications regarding the recalibration of vacuum gauges are involved.

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Radiative Recombination in Gallium Phosphide Point-Contact Diodes

CAREFUL observation of the radiation emitted from point-contact gallium phosphide diodes under conditions of reverse bias has shown that the luminescence is associated with the recombination of carriers in the breakdown region of the current-voltage characteristic. Electroluminescent radiation has been observed in gallium phosphide by several workers[1–5]. The electroluminescence appears to be associated with p–n junctions formed between the p-type bulk and an n-type skin on the surface of the material and at grain boundaries. From measurements of optical absorption in gallium phosphide, a value for the energy gap at room temperature of 2·19 eV. was deduced. This value is in good agreement with the value of 2·20 eV. for the room temperature band gap measured at Bell Telephone Laboratory[6].

We obtained a measurement of the energy spectrum of the recombination radiation by a simple and convenient arrangement of a diffraction grating and a 35-mm. camera. A diffraction grating with 15,000 lines per inch was attached to the 50-mm. extension bellows of an Exakta camera, and a 100-μ slit was affixed in the focal plane of the lens. With this arrangement, both zero-order and first-order spectra are visible on a single frame. The gallium phosphide luminescence was exposed through the slit to Ansco Super Hypan film for times up to 30 min. Fig. 1 is a print of one such exposure (×10). (The zero-order spectrum is not shown.) The two discrete lines at the left of the figure are the two prominent lines from the spectrum of gallium (4033 Å. and 4172 Å.), and are used for calibrating the spectrograph. They may be obtained by producing an arc between the point contact and the gallium phosphide crystal after the exposure of the recombination radiation has been made. Fig. 2 is a densitometer trace of the negative showing the wave-lengths and energies of the observed radiation. Correcting for the spreading effect due to the width of the slit, the energy of the recombination radiation is here observed to be between 1·96 and 2·19 eV., with a peak density at 2·12 eV. The peak

Fig. 1. First-order spectrum of recombination radiation from gallium phosphide point-contact diode

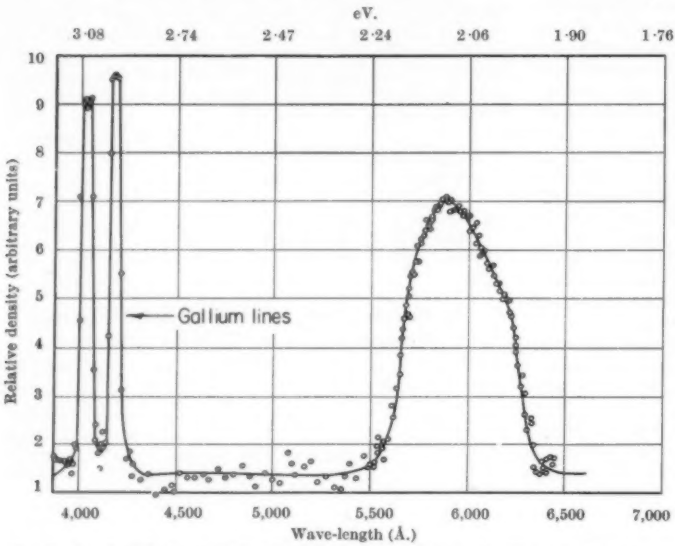

Fig. 2. Densitometer trace of the first-order spectrum of recombination radiation from gallium phosphide point-contact diode

density and minimum energy values correlate with those published by Wolff *et al.*[1]. However, interestingly enough, our maximum energy 2·19 eV. corresponds closely to our measured value of the minimum energy between the conduction and valence bands, and Wolff's maximum energy 2·32 eV. corresponds to his measured value of the band gap. Although the actual operating temperature of the diode was not determined, operation of the device in the breakdown of the reverse characteristic dissipated sufficient heat to increase its temperature significantly. Therefore, deductions concerning the probability of direct interband transitions from our results are not made, pending precise temperature measurement and control of the diode under operating conditions. The point-contact gallium phosphide diodes were prepared from small single crystals of *p*-type material, grown by the vapour-phase transport of the compound. A rather wide range of peak reverse voltages was observed, varying from 10 to 60 V. The slope of the reverse characteristic of the breakdown region also varied from diode to diode, and in some cases multiple slopes were observed.

It has been shown that in some silicon junctions, avalanche breakdown is accompanied by the emission of light at isolated points which are presumably associated with regions where dislocations pass through the junction[7]. Goetzberger and Shockley[8,9] have recently shown that the multiple slopes seen in the breakdown region of some silicon junctions are associated with isolated areas of high density of impurity which penetrate the *p–n* junction. Such a correlation between the onset of luminescence at isolated points in the breakdown region and discontinuities in the slope of the reverse characteristic was observed in our gallium phosphide junctions. The luminescent points appeared to be on the top surface of the crystal and were generally separated from the point contact, indicating that rectification was not restricted to the point contact, but was also evidently associated with a junction between an *n*-type surface skin and the bulk *p*-type material. Fig. 3 is the drawing of an oscilloscope trace of the reverse characteristic of a point-contact gallium phosphide diode. As the voltage was increased, new points of luminescence were observed to appear at the same voltages as the discontinuities in the slope of the reverse characteristic.

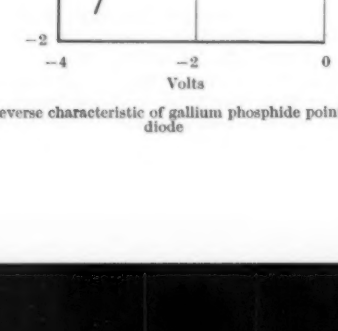

Fig. 3. Reverse characteristic of gallium phosphide point-contact diode

Appreciation is expressed to J. F. Miller for preparing the gallium phosphide and to Dr. P. W. Davis for the band-gap determination.

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Determination of Fluid Saturation in Porous Media by a Radio-Frequency Method

A KNOWLEDGE of fluid saturation at any one cross-section is very often required when studying fluid behaviour in porous media. Investigators measuring this have employed a variety of techniques including resistivity methods[1,2], radioactive tagging of a fluid[3], X-rays[4], and light absorption[5]. Very often it is required to know the extent of saturation of water or a water solution in a porous medium. Since water has a high dielectric constant, it has seemed worth while to consider a method which is dependent on this fact, and which would be used primarily for water or water solution. The technique proposed here has its origins in high-frequency titrations[6,7], where a high-frequency signal is fed into an electrode or coil on the titration cell. Changes in composition during the course of a titration are then measured in terms of change in current or voltage of the plate or grid of the oscillator valve or change in frequency of the oscillator. A change in degree of saturation in a porous medium will produce a similar loading effect in an oscillator as a high-frequency titration, and consequently, a similar method of detection may be employed.

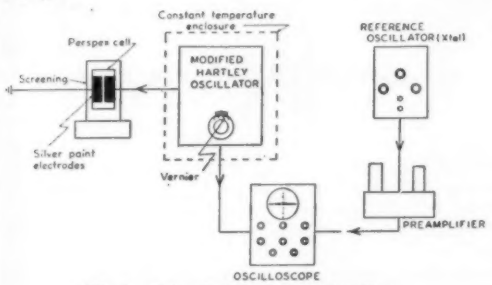

Fig. 1. Determination of fluid saturation

In the present arrangement (Fig. 1) the signal from a modified Hartley oscillator was fed into a silver paint electrode on one-half of a 'Perspex' cell (internal diameter and height 4 and 8·9 cm. respectively) and to the X-plates of an oscilloscope (Heathkit *O*-11). The electrode on the opposite side of the cell was earthed and the cell was screened by surrounding it with a copper shield. The signal from the crystal-controlled reference oscillator (a Heathkit *TO*-1 Colpitts oscillator) was sent to a 4-watt pre-amplifier (General Electric Phono-mic) and the amplified signal fed into the Y-plates of the oscilloscope. Two frequencies were used in the present work, 500 kc./sec. and 1,000 kc./sec., by selecting the appropriate crystal in the reference oscillator. It was found in the initial experiments that the oscillator was quite sensitive to temperature, and a slow frequency drift took place at room temperature. To obviate this difficulty the oscillator was enclosed in a constant-temperature cabinet.

With the cell empty, the variable oscillator was tuned to the frequency of the reference oscillator by means of a vernier on the variable capacitor of the oscillator. The point at which both signals were in phase on the oscilloscope served as an initial reference point. The cell was then packed with an unconsolidated silica sand of $-30 +40$ U.S. sieve size and the oscillator re-tuned to reference oscillator frequency. This point served as a reference point for the subsequent measurements with a fluid in the porous medium. The extent of fluid saturation of the packed cell was measured by the change in capacitance in the Hartley circuit in terms of vernier divisions, at balance, from that for the dry medium. Removal of the cell from the oscillator signal cable for unpacking could result in a capacitance change when the cell was replaced: however, with experience it was found that the initial reference point could be attained by adjustment of the position of the cable.

A series of fluid saturations was obtained by mixing a known quantity of fluid, in this case distilled water or 0·05 *N* sodium chloride solution, with a known quantity of sand and packing the resultant homogeneous mixture into the cell, *in situ*. Porosity of the medium was $34·3 \pm 0·3$ per cent for the complete range of saturations up to 100 per cent. Measurements were made of change in capacitance or frequency change in terms of difference in vernier reading (reading for cell with fluid minus reading for cell without fluid) over the range of saturations from 0 to 100 per cent.

When fluid saturations exceeded 30–40 per cent a slow frequency drift took place, always in the same direction. This was attributed to gravity drainage. When conditions had stabilized, vernier settings could be reproduced for the same fluid saturation to within less than $\pm 0·1$—the total vernier difference being of the order of 50 divisions, for 0–100 per cent fluid saturation. The overall effect of gravity drainage was to cause a difference of vernier reading of up to 0·4 of a division from the initial reading—this corresponded to a variance in the fluid saturation of up to 5 per cent. Since this distribution of fluid could not be handled quantitatively the readings for a particular fluid saturation were taken at time $t = 0$, that is, before any drainage took place. The effect of gravity drainage on the capacitance or vernier reading is illustrated for a number of fluid saturations in Fig. 2. It can be seen that the system may take as much as one hour to become stable. The curves illustrating the relationship between fluid saturation and capacitance (vernier reading difference) are shown in Fig. 3. It is apparent from the general shape of the curves that the greatest sensitivity of the method is in the regions of low saturation. In addition,

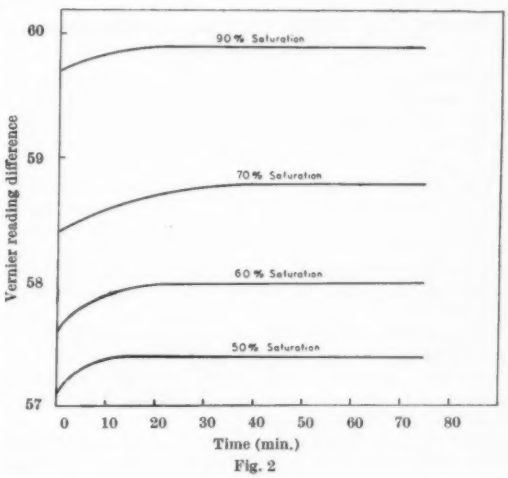

Fig. 2

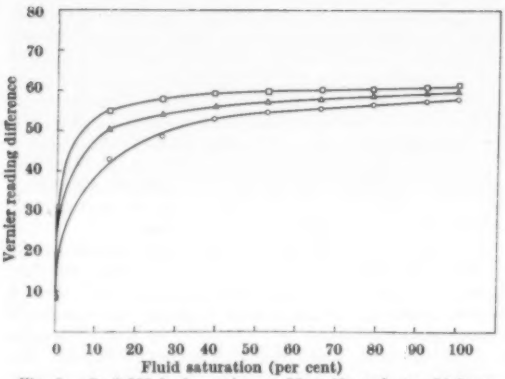

Fig. 3. ○, 1,000 kc./s., water, − 30 +40 sand, ϵ = 34·3 per cent; △, 500 kc./s., water, − 30 + 40 sand, ϵ = 34·3 per cent; □, 1,000 kc./s., 0·05 *N* sodium chloride solution, − 30 + 40 sand, ϵ = 34·3 per cent

increase of frequency appears to change the curvature, increasing the extent of the sensitive range. In the case of the 0·05 *N* sodium chloride solution the curve is displaced in such a manner as to decrease the sensitive range, and at any particular fluid saturation this is an analogous situation to a high-frequency titration, in that changes in concentration are being measured at constant saturation.

Further investigations of this method of measuring fluid saturation in porous media are continuing with the aim of increasing the range of sensitivity by the use of higher frequencies.

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GEOPHYSICS

Unidentified Earth Tremors in Dominica, West Indies

Tremors the origin of which has not been identified were recorded by our seismograph station at Roseau in the volcanic island of Dominica during September 1959, and have been recorded at irregular intervals ever since.

Each tremor consists of an approximately sinusoidal ground motion of 0·3-sec. period. The first swing is always down, the first and eighth swings are maxima and there are about twelve swings in all. The ground amplitude of these maxima varies from about 0·1μ, which is the point at which the tremors become masked by the microseismic background, to 1·0μ. They are quite unlike the tremors produced by near earthquakes or by explosions on land or at sea. Factories, quarries and other possible artificial sources for the tremors in Dominica have been investigated and eliminated.

The tremors tend to occur in bursts, numbering from two or three to several hundred tremors, which may start at any hour of the day or night and continue for periods up to twelve hours. The average period between bursts has been seven days, but there is no evidence of periodicity.

We have operated three additional Willmore–Watts seismographs in Dominica for six weeks in January and February 1960 and have found that the tremors can be recorded only within a few kilometres of the town of Roseau. Because of long quiet periods between bursts of activity, useful recordings were obtained only of a burst of five tremors at one station 1 km. from the Roseau Station and at the Roseau Station itself. There is a possible correlation between the differences in arrival times and the ratios of the maximum amplitudes of the tremors at the two stations, which is consistent with each tremor having originated at a point source and having obeyed an inverse square law of attenuation. Assuming such an attenuation law the amplitudes at the two stations indicate that the sources lie close to a circle of about 5 km. diameter, the northern part of which passes through the town of Roseau. The differences in arrival times at the stations indicate a seismic velocity between 150 m./sec. and 740 m./sec. depending on the locations of the sources on the circle.

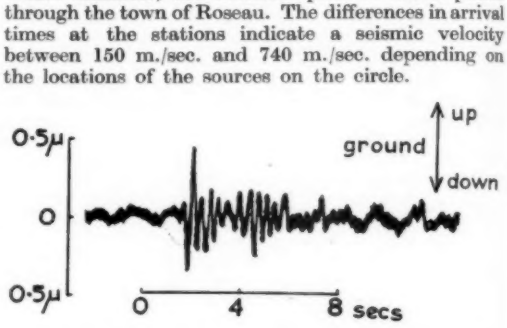

Fig. 1 Unidentified earth tremor recorded at the Seismic Research Unit's Seismograph Station at Roseau, Dominica, on September 11, 1959

The town of Roseau is situated on a delta of sand and gravel built by the Roseau River on the steeply shelving east coast of the island of Dominica. In such materials the velocity of longitudinal waves is about 750 m./sec. We have estimated the energy release at the source as about 10^{14} ergs for the largest tremor recorded. The island contains several active thermal areas and ancient centres of eruption, but none is near the apparent sources of the tremors. We can find no evidence that these are related in any way to the volcanic activity of the island; but we cannot put forward an alternative explanation for their origin. We should be grateful to hear from others who have had experience of similar tremors.

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CRYSTALLOGRAPHY

Crystal Structure of Orthorhombic Cobalt Molybdate

In petroleum refinery practice de-sulphurization is carried out by catalysts consisting of an η- or γ-type alumina impregnated with cobalt and molybdenum oxides. However, the nature of the cobalt and molybdenum compound or compounds present on

the catalyst is quite unknown, and a search through the literature reveals that there is almost no information on the formula and crystal structure of cobalt molybdate. A mineral paterite is known which is quoted as having the formula $CoMoO_4$ [1].

A series of iso-structural normal tungstates of small cations is known[2] the general formula of which is RWO_4, where R = cobalt, iron, magnesium, manganese, nickel and zinc, and recently the structure of nickel tungstate has been investigated in detail[3]. Powder diffraction patterns of laboratory and commercially prepared cobalt molybdate, after heating to 700° C., show that the compound does not possess a structure isomorphous with the tungstate family. However, normal molybdates and tungstates of larger cations, for example, barium, calcium, cadmium, lead and tin, are known and form an iso-structural series, the parent structure being scheelite.

In order to provide fundamental information, attempts were made to grow single crystals of cobalt molybdate from a melt of normal sodium molybdate, anhydrous cobalt chloride and sodium chloride as a flux in the ratio of 1 : 2 : 2 by weight. The melt was kept at 750–800° C. for 2 hr. and allowed to cool slowly in the furnace. Small yields of crystals were obtained, but the quantities available were too small for accurate analysis. The percentages by weight from chemical and spectroscopic analyses are given in Table 1, leading to an empirical formula of $Co_2Mo_3O_{11}$.

Table 1

	Cobalt	Molybdenum
Chemical	21·1	45·8
Spectroscopic	20·1	51·2

Examination of the crystals in polarized light and subsequent X-ray photographs showed that monoclinic and orthorhombic crystals were present, both forms being deep blue in colour and possessing well-marked crystal faces. The monoclinic crystals will not be reported on here. The orthorhombic crystals possessed two well-formed crystal habits which were either long thin needles up to 2 mm. in length by 0·1 mm. × 0·1 mm. cross-section, or platelets with dimensions up to 1 mm. long × 0·3 mm. wide but only 0·01 mm. thick, with a pair of edges parallel to the long dimension. X-ray photographs showed that the shortest unit cell axis a was parallel to this dimension and to the needle axis, while the longest unit cell axis c was perpendicular to the plane of the platelet. The crystals showed straight extinction, and an acute interference figure was obtained on (001). The crystals are positive with α parallel to b, β parallel to a and γ parallel to c. No attempt was made to measure $2V$ or any of the refractive indices.

Laue and symmetrical oscillation photographs about all the axes confirmed the orthorhombic symmetry, and from rotation and zero layer Weissenberg photographs using copper $K\alpha$ radiation the unit cell dimensions were calculated: $a = 5{\cdot}245 \pm 0{\cdot}003$ Å., $b = 10{\cdot}778 \pm 0{\cdot}005$ Å., $c = 18{\cdot}017 \pm 0{\cdot}006$ Å., $V = 1018{\cdot}5 \pm 2$ Å.^{3}. The error is an estimated one.

The density was found to be $3{\cdot}79 \pm 0{\cdot}05$ gm./c.c. by flotation in a solution of thallous malonate and formate, leading to four formula units of $Co_2Mo_3O_{11}$ in the cell. Theoretical density = 3·80 gm./c.c.

Zero and upper-layer Weissenberg photographs about each axis showed that all hkl reflexions were present and that systematic absences were observed for $0kl$ with $(k+l)$ odd and for $hk0$ with h odd. The space group is therefore either $Pn2_1a$ or $Pnma$. A simple test for pyroelectricity was inconclusive, but the application of intensity statistics[4] to the principal zones $hk0$, $0kl$, $h0l$ and to all hkl reflexions showed that the distributions were centric. Space group $Pnma$ was therefore adopted.

A crystal fragment 0·3 mm. × 0·15 mm. × 0·2 mm. was cut from one of the largest needles, and zero-layer Weissenberg photographs about the three axes and equi-inclination photographs of layers $h = 1$ to 8 about the a axis were taken on molybdenum $K\alpha$ radiation using the multiple film technique. The intensities of all reflexions were measured out to the maximum limit of observation ($\theta \simeq 45°$). In all 103 $hk0$, 294 $h0l$, 314 $0kl$ and 2,412 hkl non-zero independent reflexions were measured by visual comparison with a calibrated strip. The intensities were corrected for the Lorentz and polarization factors to give a set of relative $|F|^2$. No absorption correction was applied as the crystal was of optimum size $\mu = 75$ cm.$^{-1}$. Wilson's method was used for placing the relative $|F|^2$ on an absolute scale and for deriving the temperature factor. $B = 1{\cdot}29$ Å.^{2}.

Patterson projections down the a and b axes, both sharpened and unsharpened, were calculated together with the complete three-dimensional Patterson function. The calculations were made on a *Deuce* computer using a programme written by Dr. J. S. Rollett (University of Oxford). The three-dimensional Patterson function was calculated over one-eighth of the unit cell at intervals of $u = 1/20$, $v = 1/80$, $w = 1/120$ and plotted in eleven sections parallel to the bc plane. Because of a limitation in the storage on *Deuce* the number of reflexions was restricted to about 2,350 and the calculation took 8¼ hr.

The projection $P(u, w)$ showed special features in that nearly all the peaks were concentrated on the two lines $u = 0$ and $u = \frac{1}{2}$ with a very large peak, 75 per cent of the origin peak height, at $(0, \frac{1}{2})$. Some of the peaks were elongated in the u direction and only two shallow peaks existed elsewhere in the function. It was therefore clear that the cobalt and molybdenum atoms must all lie in or very close to the n glide plane. The projection $P(v, w)$ gave a well-resolved map, but no attempt was made to solve it directly and all the heavy atom co-ordinates were derived from $P(u, v, w)$.

Nearly all the peaks in $P(u, v, w)$ were concentrated in the sections $P(0, v, w)$ and $P(\frac{1}{2}, v, w)$, both of which showed apparent two-fold symmetry, about $(0, \frac{1}{4}, \frac{1}{4})$ and $(\frac{1}{2}, \frac{1}{4}, \frac{1}{4})$ respectively, of peak position but not peak height. The absence of peaks at $(0, \frac{1}{2}, 0)$, $(\frac{1}{2}, 0, \frac{1}{2})$ and $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$ clearly indicated that no heavy atoms were sited on the centres of symmetry. They are therefore either in the general positions or on the mirror plane. Co–Co, Mo–Mo, Co–Mo, Mo–O and Co–O single interaction peaks were scaled in terms of the origin peak, and from a study of all the Harker symmetry vectors for the general and special positions it was possible to derive the co-ordinates of all the heavy atoms. Eight cobalt and eight of the molybdenum atoms were sited in the general positions and the remaining four molybdenum atoms on the mirror plane. The non-Harker vectors were calculated from these co-ordinates, which gave satisfactory agreement between peak heights and positions and accounted for all the major peaks in $P(u, v, w)$.

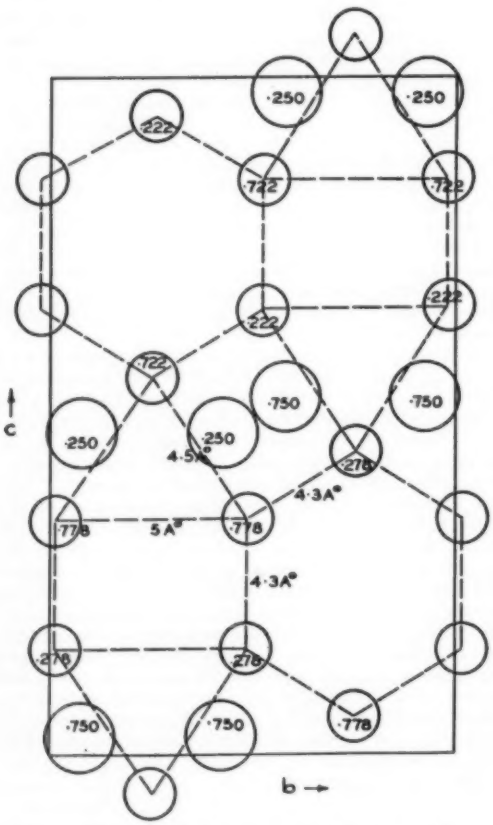
The co-ordinates are:

		x	y	z
General positions	8 cobalt	0·250	0·075	0·473
	8 molybdenum	0·278	0·017	0·155
Special positions	4 molybdenum	0·278	0·250	0·944

So far as could be detected the cobalt atoms were sited in the n glide planes with both sets of molybdenum atoms distributed a little to each side of it.

It was not possible to derive any information about the positions of the oxygen atoms as single Co–O or Mo–O peaks were not above the background of the function. Typical Mo–O distances vary between 1·75 and 2·2 Å. and Co–O distances are about 2·0 Å.; but there were no well-defined peaks around the origin peak and within these limits. A ring of high ground was present in the sections for which u = 3/20, 4/20 and 5/20. Buergers minimum function $M_2(yz)$ was applied twice to $P(v, w)$ using Co–Co and Mo–Mo rotation peaks, confirming the positions of all the heavy atoms, but the positions of the oxygen atoms were not resolved.

The unit cell projected on the 100 face is shown in Fig. 1, from which it is seen that the molybdenum atoms appear to lie at the corners of a puckered hexagon. The interatomic distances around the hexagon are almost equal at 4·3 Å. with somewhat larger distances between the hexagons of 4·5–5·0 Å. These distances are considerably larger than those found in the oxides Mo_4O_{11}, Mo_8O_{23}, Mo_9O_{26} and MoO_3, where the structure is composed of linked octahedra for which Mo–Mo = 3·25 Å. for octahedra sharing edges and 3·75–3·8 Å. for octahedra sharing corners. In unlinked octahedra Mo–Mo = 3·92–3·96 Å. It seems clear that octahedral co-ordination is not present in this structure but that free unlinked $(MoO_4)^{--}$ exist. If this is so, then there must be 48 oxygen atoms in the cell and the chemical formula must be revised to $Co_2Mo_3O_{12}$.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c
b

Fig. 1. Projection on bc plane. Large circles, cobalt; small circles, molybdenum

I wish to thank the Chairman and Directors of the British Petroleum Co., Ltd., for permission to publish this communication. Thanks are also due to Mr. L. A. Lloyd, who helped in the measurement and statistical analysis of the intensity data, and to Mr. D. Alderton, who made the calculations.

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METALLURGY

Frictional Properties of Molybdenum Disulphide Films containing Inorganic Sulphide Additives

Solid lubricants are most useful as films which separate metal surfaces that slide against each other. To be effective, the films should be highly durable. Recently, we have observed that inorganic additives can greatly enhance the durability of such films.

During the course of some work on the frictional properties of lamellar sulphides which crystallize in the cadmium iodide system, it was observed that though stannic sulphide displayed relatively high friction, it formed excellent films when rubbed over a metal surface. In such an experiment good filming is an essential property which the inorganic solid must possess if it is to lubricate effectively. When an adherent film is formed on the metal, shearing is confined to the film and the sliding behaviour is that of the lubricant against itself. It was observed that the filming properties of molybdenum sulphide, which is an excellent lubricant, could be enhanced by adding 10 per cent by weight of stannic sulphide. However, a number of additional unexpected synergistic effects were also noted. The most important of these was an improved resistance of the films to penetration under highly loaded sliding conditions. An experimental programme was initiated to find other effective sulphide additives, and some of the results are briefly summarized here.

Rider samples were prepared from powders of high-purity molybdenum disulphide, and 10 per cent by weight of finely ground sulphide additives. The solids were mixed, passed through a 200-mesh screen and compressed into $\frac{5}{16}$-in. diameter pellets at a pressure of 20,000 p.s.i. without the use of added binder. The riders were rubbed against disks of degreased and de-oxidized mild steel which had been cut from rolled sheets.

The test procedure, which used a friction dynamometer designed by E. H. Hull[1], has been described in detail[2]. Briefly, lubricant riders were rubbed against the steel at a load of 2 kgm. and a speed of 70 cm./sec.

for several hours to establish a film. Then a $\frac{1}{8}$-in. diameter chrome alloy steel hemisphere was slid over the film at the same speed. Load was increased in 50-gm. increments according to a standard schedule until film failure (metal-to-metal contact or seizure) was indicated by a precipitous rise in friction. Observations were taken in room air at 25° C. within the relative humidity range of 30–40 per cent. The seizure loads attained with a number of additives together with the minimum values of coefficient of friction for the films are reported in Table 1.

Table 1

Additive	Coefficient of friction	Maximum load carried (kgm.)	Additive	Coefficient of friction	Maximum load carried (kgm.)
Sb_2S_3	0·057	5·8	ZrS_2	0·061	2·3
PtS	0·056	5·5	HgS (black)	0·048	1·8
TiS_2	0·04	5·4	Ti_2S_3	0·060	1·5
HgS (red)	0·038	5·3	Cr_2S_3	0·08	1·2
Ag_2S	0·06	5·3	BaS	0·07	1·0
PbS	0·038	5·2	Tl_2S	0·082	0·9
FeS	0·063	4·9	SnS	0·07	0·9
Tl_2S_2	0·065	4·9	CaS	0·15	0·9
Cu_2S	0·064	3·9	CdS	0·14	0·8
CuS	0·059	3·6	FeS_2	0·40	0·3
Au_2S	0·065	2·8	ZnS	—	0·3
Bi_2S_3	0·045	2·9	MoS_2	—	0·3
SnS_2	0·047	2·8			

For the most favourable case the additive increases the seizure load for the film by more than a factor of twenty over that for a pure molybdenum disulphide film. The low friction values indicate that molybdenum disulphide is the lubricant in all cases and that the additives probably take little part in any shearing phenomena. Similar results were obtained when tungsten disulphide was the lubricant in place of molybdenum disulphide. For substrates harder than mild steel (for example, 1090 steel, chromium plate) the seizure loads observed are even greater.

The action of these additives in solid lubricants resembles that of the extreme pressure additives for liquid lubricants. A chemical role is assigned to such additives, and a similar mechanism may explain the behaviour of additives in solid lubricant films. However, evidence to support this hypothesis is indirect. Where thermodynamic data are available, the most effective additives tend to have the lowest stability. Films of molybdenum disulphide have higher load-carrying ability if they are applied to mild steel surfaces on which a surface film of ferrous sulphide has been formed by chemical reaction. Chemical analyses indicate that an active additive tends to be more concentrated in a rubbed film than in the rider from which the film was rubbed.

Though the hypothesis of chemical attack by the additives is attractive, the results do not preclude the possibility that effects other than chemical are important in enhancing the adhesion of the film to the metal substrate.

It is planned to publish a more detailed account of this work elsewhere.

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Schenectady, New York.

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RADIATION CHEMISTRY

Effect of X-Rays on Cytosine in Aerated Aqueous Solution

While X-irradiation of aqueous solutions of thymine and uracil in an atmosphere of air gives rise to high yields of hydroperoxides[1–3], X-irradiation of cytosine under similar conditions does not give more than traces of hydroperoxides in our hands.

However, ion exchange chromatography of cytosine solutions irradiated with X-rays under air or oxygen (1 atm.) separates three degradation products, which are not detected when the solutions have been irradiated under nitrogen. A typical elution diagram is shown in Fig. 1, giving the positions of these three products, designated as *A*, *B* and *C*, and of unchanged cytosine. Product *C* and cytosine are detected by measuring the optical densities of the chromatographic fractions at 300 mμ and at 275 mμ, respectively. Products *A* and *B* are not detected under these conditions, since they do not absorb strongly at these wave-lengths; but if the fractions containing them are first boiled for 30 sec., their optical densities at 275 mμ increase to give the figures plotted in Fig. 1, products *A* and *B* being transformed by heating into compounds with high absorption coefficients at this wave-length.

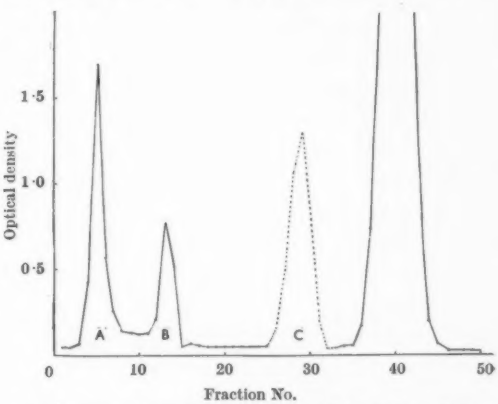

Fig. 1. Ion exchange chromatography of X-irradiated cytosine. Cytosine solution: 10^{-3} *M* in water, 40 ml., irradiated in the presence of air at room temperature. X-rays: 40 kV., filtered through 0·04 mm. aluminium; dose-rate: 25 kr./min.; dose = 150 kr. Chromatography on 'Dowex 50, H^+' (125 cm.), elution with 2 *N* hydrochloric acid at room temperature; fraction volume: 3 ml. ——, Optical density at 275 mμ; – – –, optical density at 300 mμ. Fractions 1–16 have been heated to boiling

Product *C* has been compared with synthetic 2,5-dioxo-6-aminopyrimidine (6-amino-*iso*-barbituric acid), prepared according to Johnson *et al.*[4]. The ultra-violet absorption characteristics of product *C* and of 6-amino-*iso*-barbituric acid are recorded in Table 1. The two compounds cannot be separated from each other by paper chromatography (Table 2) or by ion-exchange column chromatography. By heating product *C* in a sealed tube at 175° for 4 hr., a compound is formed having the absorption spectrum (Table 1) and the chromatographic behaviour (Table 2) of synthetic *iso*-barbituric acid, obtained by deamination of 6-amino-*iso*-barbituric acid under the same conditions[4]. All these results allow us to conclude that product *C* actually is identical with 6-amino-*iso*-barbituric acid. Under the conditions

of the experiment described in Fig. 1, the yield in product C is $G = 0{\cdot}34$.

When product A is heated to boiling in 2 N hydrochloric acid, the resulting compound has the same ultra-violet spectrum (Table 1) and the same chromatographic behaviour on paper chromatograms (Table 2) as *iso*-barbituric acid. The yield in product A, estimated after transformation to *iso*-barbituric acid, is $G = 0{\cdot}20$. When product B is similarly heated, ion exchange chromatography of the resulting solution separates two products B_1 and B_2. Product B_1, which is much more abundant than B_2, has the same ultra-violet spectrum (Table 1) and the same chromatographic behaviour (Table 2) as *iso*-barbituric acid. B_2, on the other hand, has the same spectral and chromatographic properties as 6-amino-*iso*-barbituric acid. If product B is heated at a nearly neutral *p*H, the same compounds are also produced, but the yield in the 6-amino one is increased relatively to that of the free acid. Since products A and B have no absorption bands between 250 and 300 mμ, their 4,5 bond is presumably saturated. B differs from A by having a 6-amino group, which is more easily lost than the amino group of 6-amino-*iso*-barbituric acid. It can be recalled in this respect that Green and Cohen[5] have already shown that 4,5-dihydrocytosine is very easily deaminated.

When synthetic 6-amino-*iso*-barbituric acid itself is X-irradiated in the presence of air (10^{-3} M solution receiving 300 kr.), *iso*-barbituric acid is formed, but no product B.

We think that the preceding experimental results can best be explained by assuming that products A and B have the formula shown in Fig. 2, and are related to cytosine through a hypothetical highly unstable hydroperoxide, since they are not produced in the absence of oxygen.

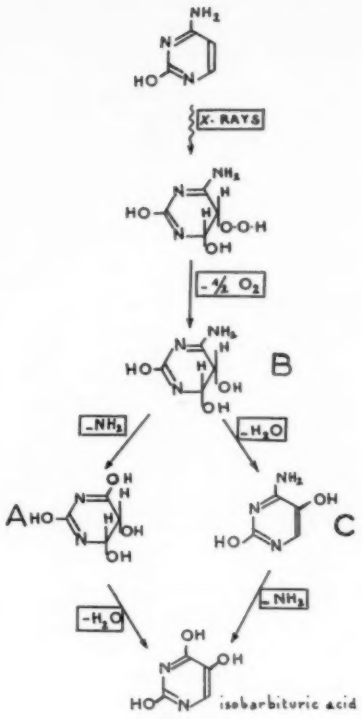

Fig. 2

Table 1. Ultra-violet Absorption Data

	*p*H 1			*p*H 11		
	Maximum (mμ)	Minimum (mμ)	$\varepsilon_{260} \times 10^{-3}$	Maximum (mμ)	Minimum (mμ)	$\varepsilon_{217{\cdot}5} \times 10^{-3}$
6-Amino-*iso*-barbituric acid	215 300	250	5·38	221 317·5	273	4·2
iso-Barbituric acid	276	246	—	307	272	—
C	215 299	250	—	221 317·5	272·5	—
C (heated at 175° for 4 hr.)	276	246	—	307	272	—
A (heated to boiling)	275	246	—	307	272·5	
B (heated to boiling) B_1	276	246	—	307	272·5	
B_2	215 300	250	—	221 317·5	272·5	

Table 2. R_F of Compounds on Paper Chromatograms*

	n-Propanol/water (10 : 3)	*iso*-Butyric acid/water/conc. ammonia (100 : 52 : 1·12)	*iso*-Propanol/water/conc. hydrochloric acid (65 : 18·3 : 16·7)
Uracil	0·53	0·62	—
Cytosine	0·42	0·73	0·55
6-Amino-*iso*-barbituric acid†	0·37	0·67	0·50
iso-Barbituric acid	0·43	0·43	—
6-Aminobarbituric acid	0·87	—	0·91
C	0·37	0·67	—
C (heated at 175° for 4 hr.)	0·43	—	—
A (heated to boiling)	0·43	0·41	—
B (heated to boiling) B_1	0·43	0·44	
B_2	0·37	0·73	—

* Development by gravity on Whatman No. 1 paper.
† Gives a blue fluorescence when irradiated with λ 2537 Å.

We wish to thank Dr. R. Latarget for his interest throughout this work.

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CHEMISTRY

Use of Chlorine in the Detection of Compounds on Paper Chromatograms

Rydon and Smith[1] described a sensitive method for the detection of amino- and imino-compounds on paper chromatograms: the method consisted of exposure of the dried chromatograms to an atmosphere of chlorine, followed by aeration to remove 'background' chlorine, and detection of the resultant chloramines by liberation of iodine from a starch/

potassium iodide reagent. In Reindel and Hoppe's modification[2] of the procedure, the chromatograms were moistened with an acetone/alcohol mixture and exposed to gaseous chlorine in a tank before detection of the chloramino derivatives with a tolidine/potassium iodide reagent. In a further modification[3], the chromatograms were moistened with steam, exposed to gaseous chlorine, heated briefly to 50° and developed with the tolidine/potassium iodide reagent.

In our hands, both the chlorination and aeration stages of the original procedure[1] proved difficult to standardize. Prolonged aeration, which had a variable effect on the chloramino derivatives, was sometimes required to remove background chlorine. It seemed that this was necessary when the tank atmosphere was particularly humid; on these occasions, and in Smith's procedure[3], the paper presumably retained a layer of chlorine water which had to be removed by aeration, or by heating to 50°, in order to obtain a satisfactory background. The need for water in the chlorination process was suggested by unsuccessful attempts to detect nitrogenous compounds using dry carbon tetrachloride saturated with chlorine as the chlorinating reagent (unpublished results).

In preliminary attempts to replace the inconvenient tank procedure by a reliable spray reagent, different effects were observed, which were used to advantage in the procedures to be described. The spray reagents were prepared by dilution of a sodium hypochlorite solution (10–14 per cent w/v) or a potassium chlorate/hydrochloric acid reagent[4], and were applied to dried chromatograms using about 1 ml./50 cm.^{2}. In procedure (*a*) the chromatogram was sprayed with sodium hypochlorite solution diluted 1 : 150, and then left for 45 min. at room temperature. In procedure (*b*) the chromatogram was sprayed with sodium hypochlorite solution diluted 1 : 25, left 45 min. at room temperature and heated for 5 min. at 55°. In procedure (*c*), the chlorate/hydrochloric acid reagent was diluted 1 : 10 and, after spraying, the chromatogram was left for 45 min. at room temperature before heating for 15 min. at 55°. Chromatograms treated in this way were developed by immersion in a tolidine/potassium iodide reagent[2].

The effect of these procedures on a variety of sugar derivatives is shown in Table 1. It can be seen that procedure (*a*) located only amino-compounds, whereas procedure (*b*) located both amino- and imino-compounds. This provided a convenient method for distinguishing amino- and imino-compounds which was particularly useful with compounds reacting poorly with ninhydrin (for example, the anomeric 1 : 3 : 4 : 6-tetra-O-acetyl-2-amino-2-deoxy-D-glucose hydrochlorides, unpublished results).

Table 1. Detection of Compounds on Paper Chromatograms using Various Chlorination Procedures and a Tolidine/Potassium Iodide Reagent (ref. 2)

Compound*	Colour of spot obtained from compounds by procedure : *a*	*b*	*c*
D-Glucose	—	—	white
Phenyl β-D-glucoside	—	—	white
2-Amino-2-deoxy-D-glucose hydrochloride	blue	blue	—
Phenyl 2-amino-2-deoxy-β-D-glucoside	blue	blue	white
2-Acetamido-2-deoxy-D-glucose	—	blue	—
Ethyl 2-acetamido-2-deoxy-β-D-glucoside	—	blue	—
Phenyl 2-acetamido-2-deoxy-β-D-glucoside	—	blue	white
Background colour	white	pale blue	blue

* 100 μgm. was used, but less is detectable in each case.

On chromatograms developed immediately after spraying with hypochlorite, the blue spots generated by the chloramino derivatives could be distinguished only with difficulty from the dark background. The success of procedures (*a*) and (*b*) depended on being able to reduce this background colour without affecting seriously the chloramino derivatives. Certain compounds, however, showed as white spots against a blue background, indicating the removal of hypochlorite and the formation of products unable to react with the tolidine reagent. It appeared that, in contrast to tank chlorinations, the amount of chlorinating agent in the spray reagents was the limiting factor. Procedure (*c*), which used the more easily controllable chlorate/hydrochloric acid reagent to generate the background colour, was based on this effect. Table 1 shows that whereas compounds with a benzene nucleus or a reducing group (for example, phenyl β-D-glucoside, glucose) were detected, the result given by compounds containing one of these groups together with an amino- or imino-group depended on the reactivity of the groups present: the results in Table 1 indicated reactivities in the order phenyl > amino > imino > reducing group. Procedure (*c*) was the most sensitive method for the detection of some compounds, particularly the anomeric phenyl 2-acetamido-2-deoxy-D-glucosides[5].

Other chlorinating agents (for example, 'Chloramine-*T*', chlorine water) and other indicator systems (for example, starch/potassium iodide, diphenylamine, *o*-phenanthroline) were tried, but none proved so satisfactory as those described. The three procedures are reproducible and can be used for the detection of a wide range of nitrogenous, aromatic and reducing compounds on paper chromatograms. However, care may be necessary in the selection and removal of chromatographic solvents, since some of these may interfere with the detection procedures (cf. Reindel and Hoppe[2]).

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A Split-Temperature Column System for Gas Chromatographic Analyses

This communication describes a simple but effective improvement on the speed and resolution in gas chromatographic analyses by means of a split-temperature column system which has provisions for two or more different temperature regions in the same unit and which provides a means of changing rapidly the temperature in these various regions (patent applied for). Such a system possesses the combined advantages of a single low-temperature system, a single high-temperature system, as well as a temperature-programmed system[1], and, usually,

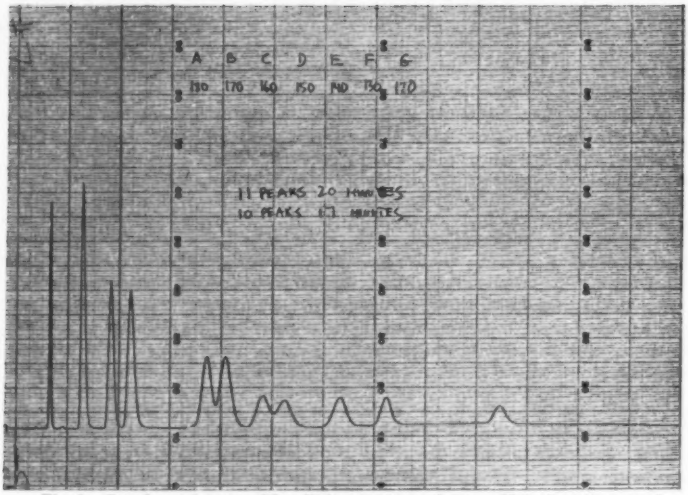

Fig. 1. Gas chromatogram with split-temperature column. Note short time, good resolution and well-defined peaks

can result in an appreciable reduction of the time required for each analysis.

Consider the analysis of a mixture containing both high- and low-boiling components. If a single low-temperature column is used, the time required to resolve the low-boiling components is sometimes unnecessarily long without any gain in resolution, whereas the high-boiling components are either held back even after a very long time, and thus cannot be detected at all, or are detected only as extremely broad peaks which cannot be accurately measured. If only a high-temperature column is used, the high-boiling components can form well-defined peaks; but the resolution of the low-boiling materials may become too poor and not useful. A manually temperature-programmed column requires considerable attention by the operator, and can give reproducible retention results only if the analysis is carried out with great care. A linear temperature-programmed system is, at least for the present, still rather expensive, and also requires longer time for analysis than the split-temperature system.

In our preliminary experiments, we used a steel enclosure consisting of seven sections individually heated by heating tapes and controlled by bimetallic thermo-regulators. These sections were partly insulated from each other by glass wool packings, and partly connected with each other by metal strips, providing thereby a sufficiently uniform temperature within each section, and a reasonably smooth temperature gradient between sections. A 10-ft. glass column, packed with 'Apiezon-*L*' on firebrick, and connected to a 'Burrell Kromotog-*K*-1', completed the chromatographic system. A mixture, consisting of (i) *n*-pentane, (ii) benzene, (iii) methyl *cyclo*hexane, (iv) toluene, (v) *o*-xylene, (vi) *m*- and *p*-xylene, (vii) ethyl benzene, (viii) *n*-propyl benzene, (ix) *i*-propyl benzene, (x) *t*-butyl benzene and (xi) *t*-butyl toluene, formed the analytical sample. In operation, portions of the column were kept initially at lower temperatures so that there would be adequate resolution for the low-boiling components, and the remainder of the column was kept at the higher ultimate temperature. After the lower-boiling components had been eluted, the whole column was brought to the ultimate temperature for the analysis of the higher-boiling substances.

A series of experiments with the various sections having different initial temperatures ranging from 120° to 180°, and an ultimate temperature of 180°, have been carried out. For the purpose of comparison, the column was also heated uniformly at 130° and at 180° only, and the corresponding chromatograms were also obtained. It was found that, for the case when the column was heated uniformly at 130° C., although the low-boiling components (i–vi) were satisfactorily resolved, the peaks for the higher-boiling components (vii–x) were ill-defined and that the peak for the highest boiling component (xi) was not detectable within a period of 1½ hr. For the case when the column was heated uniformly at 180° C., the total time required for the 11 components was 14 min.; but the resolution of components (v) and (vi) and components (vii) and (viii) was very poor and not useful. In contrast, the split-temperature column system permitted the separation of all eleven components in a time-period of 20 min. with the resolution of the low-boiling components comparing favourably with those obtained by the low-temperature column, and the resolution of the high-boiling components comparing favourably with those obtained by the high-temperature column. This is illustrated in Fig. 1, which shows clearly the satisfactory performance of the split-temperature system from the points of view of both the short time required for analysis and satisfactory resolution.

A split-temperature column system can be constructed easily and inexpensively with readily available components, and should be usable with most of the existing gas chromatographs. It permits the analysis of a larger number of samples per chromatograph, and also permits the use of one single long column for each type of packing since the effective length of a column now can be conveniently varied by changing the length of the lower-temperature portions of the column.

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Separation of Sphingosine and Dihydrosphingosine by Reversed Phase Paper Chromatography

The isolation of dihydrosphingosine from cerebrosides of beef brain and spinal cord by Carter *et al.*[1] raised questions regarding its distribution in different sphingolipids and its metabolic relationships to sphingosine. However, no method for the routine

Fig. 1. Chromatogram showing the dinitrophenyl derivatives of O-methylsphingosine (1), dihydrosphingosine (2), sphingosine (3) and crude base fraction (*CB*). (4) is dinitrophenol

differentiation of sphingosine and dihydrosphingosine appeared until very recently with the gas chromatographic method of Sweeley and Moscatelli[2]. Wittenberg[3] separated the N-succinyl derivatives of sphingosine and related compounds by reversed phase chromatography on diatomaceous earth treated with dichlorodimethylsilane, but he was unable to separate sphingosine and dihydrosphingosine. This communication describes a reversed-phase paper chromatographic method for the separation of the dinitrophenyl derivatives of sphingosine and dihydrosphingosine.

A crude fraction of sphingolipid long-chain bases was prepared principally according to Carter *et al.*[1]. 1–5 mgm. of the bases were transferred to their dinitrophenyl derivatives by keeping them for 30 min. in 4 ml. of a potassium borate buffer, *p*H 10, containing 2 μl. fluorodinitrobenzene in 1 ml. ethanol at 60° C. The derivatives were extracted with diethyl-ether and 5–15 μgm. were applied with a micropipette to Whatman paper 3*MM* on a line 2 cm. from the bottom of the paper. The paper was sprayed almost to transparency with the stationary phase of tetralin down to a line 1 cm. above the line of the derivatives. The paper was then immediately run in methanol/triethylamine/water (85 : 5 : 10 v/v) saturated with tetralin in the ascending direction for 17 hr. at room temperature. The spots were read under ultra-violet light and the R_F values obtained were for dinitrophenyl-O-methylsphingosine 0·20, dinitrophenyl-dihydrosphingosine 0·36, dinitrophenyl-sphingosine 0·44 and dinitrophenol 0·87 (Fig. 1). The R_F values of dinitrophenyl-O-methylsphingosine and dinitrophenyl-O-methyldihydrosphingosine differed only little and the spot of dinitrophenyl-O-methylsphingosine therefore also contained dinitrophenyl-O-methyldihydrosphingosine. Dinitroaniline, which was only just traceable, had an R_F value of 0·71. Authentic samples of triacetylsphingosine, tribenzoyldihydrosphingosine and O-methyl-sphingosine sulphate, which were gifts from Dr. Carter, University of Illinois, were after hydrolysis used as references for the identification of the bases.

These were further indirectly identified by the following procedures. The dinitrophenyl-O-methyl-sphingosine spot disappeared after changing the hydrolysis in the isolation procedure from methanolic to aqueous sulphuric acid. Sphingosine and dihydrosphingosine were precipitated from the base fraction according to Carter *et al.*[4]. After hydrogenation of the bases with platinum oxide as catalyst the dinitrophenyl-sphingosine spot disappeared and the dinitrophenyl-dihydrosphingosine spot was correspondingly enlarged.

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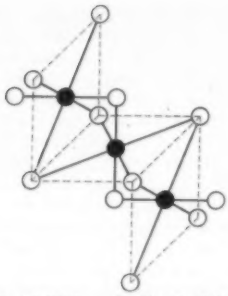
Structure of the Trinuclear Titanium (IV) Alkoxides

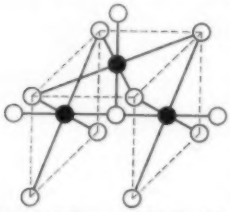
THE association of *n*-alkoxy derivatives of orthotitanic acid was first established as recently as December 1951 by Caughlan *et al.*[1]. Cryoscopic determinations of molecular weight in freezing benzene revealed that $Ti(OC_3H_8)_4$, $Ti(OC_3H_7)_4$ and $Ti(OC_4H_9)_4$ were all trimeric except at very high dilutions when dissociation to the monomer was observed. The association was ascribed to the strong tendency of quadrivalent titanium to attain a six-covalent oxygen environment, as, for example, in rutile or anatase. Caughlan *et al.* suggested that this could be achieved if each pair of titanium atoms was supported by three bridging alkoxy-groups giving two alternative structures I and II, which were based on the stacking of three TiO_6-octahedra face to face. Structure II necessitates a four-co-ordinated square planar oxygen atom attached to three titanium atoms and one alkyl group, and for this reason is considered by us to be untenable.

Structure I has been widely accepted[2] as the basis on which to interpret the chemistry of titanium (IV) alkoxides, and, in particular, the group working at Birkbeck College[3] have stressed this arrangement when discussing the hydrolysis of these compounds. In fact, they have claimed that the synthesis of polymeric oxide alkoxides (structure IV), built up by cross-linking trimer units with structure I through Ti-O-Ti bridges, "appears to be unique and justifies exclusive consideration".

The purpose of this communication is to indicate that at least two other stereochemical arrangements are possible, which we believe are in better agreement with the available experimental data. The first of these is again based on a six-covalent oxygen environment for titanium, but a trigonal prismatic rather than an octahedral arrangement of the TiO_6-group is envisaged. This leads to structure III in which two 'bridge' and two 'terminal' alkoxy-groups per



(I) $Ti_3(OR)_{12}$. ●, Ti; ○, O in OR



(II) $Ti_3(OR)_{12}$. ●, Ti; ○, O in OR

titanium atom are present. A geometrical model reveals that twelve *n*-alkyl groups can be nicely accommodated about the three titanium nuclei without steric hindrance. We believe that an important advantage of structure III lies in its natural symmetry, in contrast with structure I, in which the central titanium atom is surrounded by six alkoxy bridges with the other titanium atoms each carrying three terminal and three alkoxy bridges. The need for a high degree of symmetry in these compounds is reflected, for example, in the interpretation of the molecular complexity of chlorine-substituted alkoxides. Thus, in agreement with Caughlan *et al.*, we also find that monochloro-titanium (IV) butoxide is trimeric. A symmetrical structure for $Ti_3Cl_3(OC_4H_9)_9$ based on structure I is not possible, but follows naturally from structure III, which can accommodate one terminal chlorine atom per titanium. Similarly, the hydrolysis reactions of titanium (IV) alkoxides become much more amenable to a reasonable kinetic interpretation. For example, the careful studies of Bradley, Gaze and Wardlaw[4] have resulted in the isolation of $Ti_4O_4(OR)_{16}$ as the product of the first stage of hydrolytic polymerization of $Ti_3(OR)_{12}$, for which they propose structure IV. A polymerization of this kind would appear to require a statistically unfavourable mutual orientation of two $Ti_3(OR)_8(OH)_4$ units before effective collision and expulsion of four water molecules could occur, and would also require the preferential hydrolysis of two terminal and two bridging alkoxide groups from only one side of the trimer. Furthermore, it also involves an unknown four-covalent bonding arrangement between oxygen and its nearest four titanium neighbours.

We suggest that the hydrolysis of $Ti_3(OR)_{12}$ with structure III can be formulated without raising the above difficulties, and yet can lead logically to oxide-alkoxide polymers of the type $Ti_6O_4(OR)_{16}$, $Ti_9O_8(OR)_{20}$ and $Ti_{12}O_{12}(OR)_{24}$ reported by the Birkbeck school. The hydrolysis of terminal alkoxy groups, the intermolecular elimination of alcohol, and the intramolecular elimination of water appear to be likely successive stages. Such a mechanism involves a kinetically simple two-point collision, and a possible scheme would be as follows (b = bridge; t = terminal group):

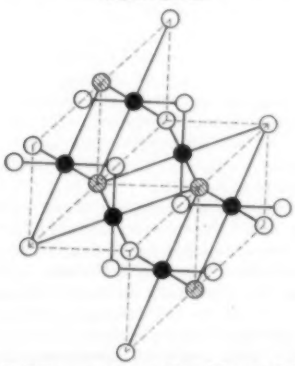
(i) $Ti_3(OR)_6{}^b(OR)_6{}^t + 6H_2O \rightarrow Ti_3(OR)_6{}^b(OH)_6{}^t + 6ROH$

(ii) $Ti_3(OR)_6{}^b(OH)_6{}^t + Ti_3(OR)_6{}^b(OR)_6{}^t \rightarrow (RO)_4{}^t(RO)_6{}^bTi_3(O)_2{}^bTi_3(OR)_6{}^b(OH)_4{}^t + 2ROH$

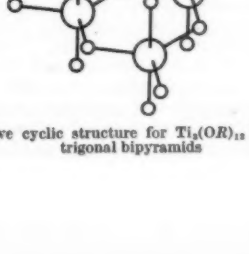
(iii) $(RO)_4{}^t(RO)_6{}^bTi_3(O)_2{}^bTi_3(OR)_6{}^b(OH)_4{}^t \rightarrow (RO)_4{}^t(RO)_6{}^bTi_3(O)_2{}^bTi_3(OR)_6{}^b(O)_2{}^t + 2H_2O$

The terminal Ti = O groups formed involve five-covalent titanium, and in this connexion the recent work of Andersson and Wadsley[5] is particularly relevant, since these workers have established that quadrivalent titanium in the mixed oxide $K_2Ti_2O_5$ is five-covalent. In a personal communication, they have suggested to us structure V, which is based on a trigonal bipyramidal TiO_5-grouping, as an alternative configuration for $Ti_3(OR)_{12}$. This differs from structure III, not only in the five-covalency of titanium, but also in that only one alkoxy-bridge per titanium atom is present.

(III) Proposed cyclic structure for $Ti_3(OR)_{12}$ based on TiO_6-trigonal prisms



(IV) $Ti_4O_4(OR)_{16}$. ●, Ti; ○, O in OR; ●, O in Ti-O-Ti



(V) Alternative cyclic structure for $Ti_3(OR)_{12}$ based on TiO_5-trigonal bipyramids

We believe that the known behaviour of titanium (IV) alkoxides and the chloro-alkoxides is in better agreement with a cyclic than a linear arrangement of the three titanium atoms. Whether the titanium is five- or six-covalent must rest on a complete structure analysis. It is quite conceivable that in the pure state or in benzene solution, titanium exhibits five-covalency, but under the influence of a strong solvating medium such as butanol, expansion to six-covalency takes place.

A more detailed account of this work will be published elsewhere.

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Heat Treatment of Irradiated Polypropylene

It has been shown by Lawton[1,2] that during the high-energy irradiation of a polymer, the main effect produced is either intermolecular cross-linking or degradation of the molecular chain, and radicals produced during irradiation can be trapped in the crystalline regions. Crystalline 'Marlex-50', irradiated at 25° C. and then annealed at 150° C., gave more cross-links than 'Marlex-50' irradiated at 25° C. and not annealed, but less than that of the polymer irradiated at 150° C. for the same dose of irradiation. We have been able to demonstrate the delayed cross-linking effect by heat treatment of pre-irradiated isotactic polypropylene. We find that many trapped radicals produced during irradiation, which are stable at room temperature (as detected by electron spin resonance measurements), could contribute to delayed cross-linking on annealing the irradiated polymer after irradiation, and a big increase of gel content was observed. On the other hand, materials irradiated at 180° C. (molten state) gave no gel content for high dosage beyond 80 Mrads, indicating that in the molten state degradation of the molecular chain was predominant. The phenomenon is very different from the effect in polyethylene.

Isotactic polypropylene ('Moplen') powder was used. The molecular weight determined from viscosity was $2{\cdot}7 \times 10^5$. The degree of crystallinity is reported to be 75 per cent. Rods were prepared by melting the powder in the glass ampoule under high vacuum. The materials were irradiated at 25° C. (in the crystalline state) and at 180° C. (in the molten state) in glass ampoules under a vacuum of less than 10^{-5} mm. mercury with γ-rays from a 1,000-curie cobalt-60 source; the irradiation intensity was $3{\cdot}0 \times 10^5$ rads/hr.

In some experiments, polypropylene powder was irradiated in air. The polypropylene rods pre-irradiated *in vacuo* were annealed for an hour in a vacuum at the temperature-range 100–230° C. in an oil-bath soon after irradiation without exposing them to air. The powder irradiated in air was annealed at 150° C. for an hour in a vacuum.

Solubility measurement to determine gel content was used as a measure of the relative cross-linking effectiveness of the irradiation. The cross-linking gel fraction was determined by measuring the insoluble fraction extracted by suspending the irradiated samples (or samples annealed after irradiation) in a large volume of boiling tetraline at 207° C. for 48 hr. After extraction, the materials were immersed in hot methanol for different times, and traces of solvent in the materials were removed, vacuum dried and weighed.

In the case of isotactic polypropylene, the delayed cross-linking effect by heat treatment of pre-irradiated polymer is very conspicuous (Table 1).

Table 1. Gel Content (per cent) of Irradiated Polymers

Irradiation dosage	13 Mrads	83 Mrads
Irradiation at 25° C. *in vacuo*	0	47
Irradiation at 180° C. *in vacuo*	0	0
Irradiation at 25° C. in air	0	0
Irradiation at 25° C. *in vacuo*, and then annealed at 180° C. *in vacuo*	34	72

The gel content of irradiated polymers under various conditions is shown in Table 1. Irradiation dosages were 13 Mrads or 83 Mrads. The gel content of the specimen annealed at 180° C. after irradiation was much higher than that of unannealed.

The observations indicate that trapped radicals in the crystal could move to form intermolecular cross-links. When irradiated at 180° C. (amorphous molten state), the specimen gave no gel content with even 83 Mrads, indicating that scission of the main chain is more important here than cross-linking.

In Fig. 1 the relations of the delayed cross-linking effect to annealing temperature in the case of pre-irradiation dosages of 13 Mrads and 83 Mrads are shown. In both cases, gel content increases rapidly in the temperature range of the melting point, due to the mobility of the trapped radicals in the crystal. The relation of the sol fraction S of specimen irradiated at 25° C. in vacuum, and of the specimen irradiated at 25° C. in vacuum and then annealed at 150° or 180° C. *in vacuo*, to irradiation dosages is shown in Fig. 2.

Charlesby's formula[3] can be applied to the results:

$$S + \sqrt{S} = p_0/q_0 + 1/q_0 U_1 R$$

in which p_0 is the probability of degradation of a molecular chain by unit irradiation, q_0 is the probability of cross-linking by unit irradiation, U_1 is number average degree of polymerization, and R is irradiation dosage (in megarads). Assuming the initial

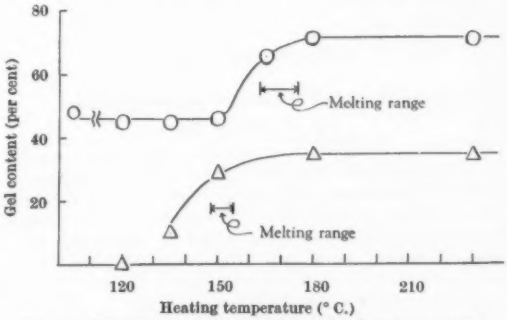

Fig. 1. Gel content of specimens annealed at various temperatures. Dosage: ○, $8{\cdot}7 \times 10^7$ rads; △, $1{\cdot}3 \times 10^7$ rads

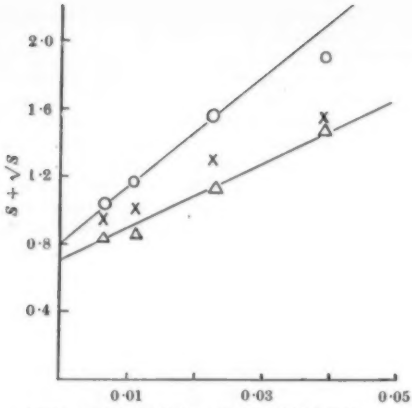

Fig. 2. ○, Irradiated at 25° C.; ×, irradiated at 25° C. then annealed at 150° C.; △, irradiated at 25° C. then annealed at 180° C.

distribution is random, the ratio p_0/q_0 and cross-linking efficiency G_c (number of cross-linking per 100 eV. absorbed) could be calculated from the results.

In the case of irradiation at 25° C., p_0/q_0 was 0·8, and G_c was 0·1, and in the case of annealing at 180° C. after irradiation, p_0/q_0 was 0·7 and G_c was 0·16. During pre-irradiation in the air, a considerable amount of peroxide[3] might be formed in the polymers. By annealing the pre-irradiated materials at 150° C., these peroxides are decomposed into the radicals which produce intermolecular cross-linking.

Table 2. Gel Content of the Materials Pre-irradiated in the Air and then Annealed at 150° C. *in vacuo*

Irradiation dosage (Mrads)	Gel content (per cent)
18	6·0
29	12·1
52	10·2
102	0

In Table 2 the gel content of annealed material pre-irradiated in the air for various dosage is shown. The gel content decreases beyond 50 Mrads because of rapid decrease of molecular weight during pre-irradiation in the air.

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BIOCHEMISTRY

Oxidation of Human Hæmoglobin by Vitamin K_3

Previous work in this laboratory has given support to the conclusion that the brain lesion in neonatal bilirubin encephalopathy or kernicterus is primarily due to a toxic effect of free bilirubin on cellular energy metabolism[1]. Hæmolytic diseases in new-born children, resulting in severe hyperbilirubinæmia, may therefore lead to the development of kernicterus, regardless of the cause of the hæmolysis.

Among exogenous factors inducing hæmolysis in new-born babies, vitamin K_3 or menadione (2-methyl-1,4-naphthoquinone), and some of its water-soluble derivatives (for example, synkavit), have recently been paid particular attention. These compounds are widely given to new-born children, and especially to premature infants, in order to improve their capacity to maintain adequate prothrombin titres. Zbinden *et al.*[2], who have recently reviewed the pertinent literature, have shown that injection of sublethal doses of vitamin K_3 into dogs leads to severe hæmolysis, accompanied by hæmoglobinuria and hæmoglobinæmia. No such effects were obtained with vitamin K_1.

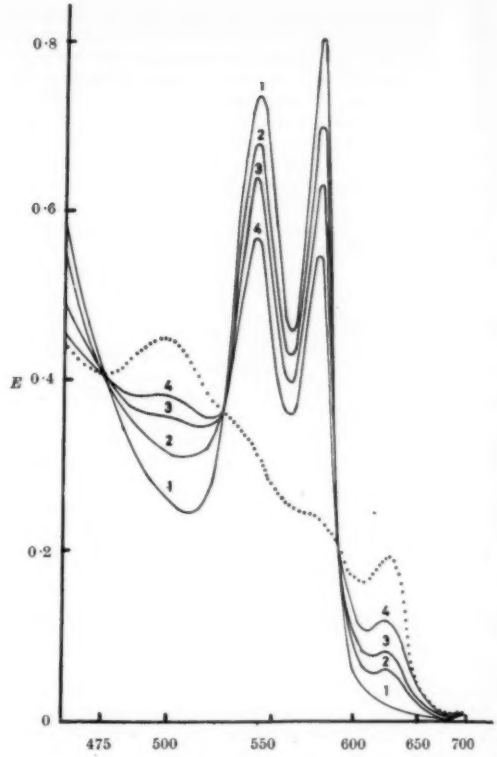

Fig. 1. Oxidation of human hæmoglobin by vitamin K_3
0·05 mM hæmoglobin in 0·05 M potassium phosphate buffer, pH 7·5.
Curve 1, before addition of vitamin K_3;
Curve 2, 5 min. after addition of 1·3 mM vitamin K_3;
Curve 3, 10 min. after addition of 1·3 mM vitamin K_3;
Curve 4, 42 min. after addition of 1·3 mM vitamin K_3;
Dotted curve, after addition of an excess of potassium ferricyanide.
Vitamin K_3 was dissolved in ethanol and added in a volume of 0·1 ml. to a final volume of 3 ml.
The measurements were carried out in a Beckman *DK*2 recording spectrophotometer, using glass cuvettes of 1 cm. light-path

In the present communication it is shown that vitamin K_3, but not vitamin K_1, oxidizes human hæmoglobin to methæmoglobin *in vitro*. It was observed that samples of whole blood, suspensions of washed erythrocytes, or erythrocyte-hæmolysates, when incubated in the presence of moderate concentrations of vitamin K_3, soon turned dark-brown, whereas vitamin K_1 induced no similar shift in colour. The phenomenon was afterwards investigated by adding vitamin K_3 to a solution of crystalline human hæmoglobin prepared according to Drabkin[3], and following the colour-shift with repeated scanning in a Beckman *DK*2 recording spectrophotometer. As shown in Fig. 1, the spectral change consisted of the

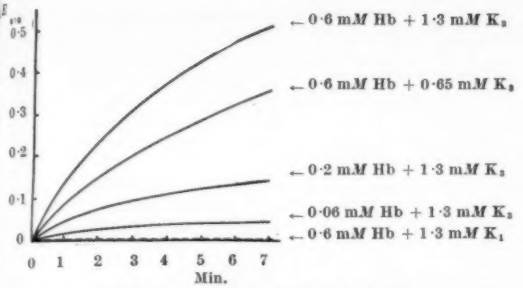

Fig. 2. Time-course of oxidation of hæmoglobin by vitamin K_3. Comparison of effects of vitamin K_3 and vitamin K_1. Hæmoglobin (Hb) and vitamin K_3 or K_1 were added in concentrations as indicated. Other experimental conditions as in Fig. 1. Vitamin K_1 was dissolved in ethanol and the solution was mixed with a small amount of 'Tween-20' to prevent separation of the vitamin K_1 on addition to the test system

appearance of an absorption maximum at 630 mμ, a decrease of the two maxima at 577 and 541 mμ, and a shift of the absorption minimum at 510 mμ toward a maximum at 500 mμ, all these changes being typical of the transition of oxyhæmoglobin into methæmoglobin[4]. The dotted line in the figure shows the spectrum of 100 per cent methæmoglobin, obtained by adding an excess of potassium ferricyanide to the cuvette.

As is already seen from Fig. 1, the reaction was not linear with time, the rate of methæmoglobin formation decreasing on prolonged incubation. The kinetics of the reaction were studied in more detail by measuring the increase in optical density at 630 mμ as a function of time. Fig. 2 shows that the rate of reaction is dependent on both the concentration of hæmoglobin and the concentration of vitamin K_3. From the curves in Fig. 2 it can be estimated that $1{\cdot}3 \times 10^{-3}$ *M* vitamin K_3 oxidizes roughly 25 per cent of the hæmoglobin present in 7 min. A more detailed study of the kinetics of the reaction was complicated by the reoxidation of the reduced vitamin K_3 by molecular oxygen, with the formation of hydrogen peroxide. Addition of substantial amounts of reduced vitamin K_3 to oxyhæmoglobin caused an immediate colour-shift due to the removal of oxygen, followed by a gradual oxidation of the hæmoglobin to methæmoglobin largely according to the kinetics shown in Figs. 1 and 2. Synkavit, the diphosphate ester of reduced vitamin K_3, had no effect on hæmoglobin under the present experimental conditions. However, this does not exclude a possible effect *in vivo*, where synkavit may undergo enzymic dephosphorylation.

It is also shown in Fig. 2 that vitamin K_1 caused no oxidation of hæmoglobin, even when added in a concentration that in the case of vitamin K_3 gave a relatively rapid oxidation.

Although the relevance of these findings to the problem of the hæmolytic effect of vitamin K_3 may not be obvious, there might exist some relation between the two phenomena. It is generally considered that hæmolysis is in some way related to a deficient ability of the erythrocyte to maintain its normal level of reduced glutathione[5]. Methæmoglobin has been reported to oxidize reduced glutathione non-enzymically[6]. Furthermore, the reduction of glutathione in the erythrocyte is mediated by glutathione reductase, with reduced triphosphopyridine nucleotide as the specific hydrogen donor[7]. This latter compound is also utilized in the erythrocyte for the reduction of methæmoglobin, by way of a reduced triphosphopyridine nucleotide-specific methæmoglobin reductase[8]. An increased rate of formation of methæmoglobin, for example, due to vitamin K_3, may therefore constitute an increased competition for reduced triphosphopyridine nucleotide, thus rendering difficult the maintenance of glutathione in the reduced form. In fact, Zinkham and Childs have reported that incubation of neonatal and of primaquine-sensitive erythrocytes with vitamin K_3 resulted in a serious decrease of the level of reduced glutathione[9].

The above hypothesis is not in conflict with the often-emphasized fact that hæmolysis and methæmoglobinæmia do not necessarily go hand-in-hand[10], since the capacity of the methæmoglobin reductase system may be sufficient for maintaining a normal level of hæmoglobin even at an enhanced rate of hæmoglobin oxidation; besides, this reaction seems not to be fully dependent on reduced triphosphopyridine nucleotide, there being also a diphosphopyridine nucleotide-specific methæmoglobin reductase[11]. Glutathione reduction, on the other hand, may seriously suffer in capacity when methæmoglobin formation, and thus the drainage of reduced triphosphopyridine nucleotide, is increased, especially in cases, as in new-born children or in primaquine-sensitive patients, where the capacity to maintain the level of reduced glutathione seems to be insufficient.

We wish to thank Dr. Andrew Sass-Kortsak, Toronto, for stimulating discussions, and the F. Hoffmann-La Roche and Co., A.-G., Basel, for the preparations of vitamin K used in this work.

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2-Hydroxyœstrogens

Recent work in several laboratories indicates that 2-hydroxylated œstrogens may be important metabolites of these naturally occurring steroid hormones. Thus King[1,2] has shown that œstriol is converted into 2-hydroxyœstriol and 2-methoxyœstriol by incubation with rat liver preparations in the presence of molecular oxygen. Work on the oxidation of di- and triphosphopyridine nucleotides by phenolases with oxygen, induced by natural and synthetic phenolic

œstrogens[3], clearly demonstrates that *o*-diphenols are involved in this system. Mueller[4] has claimed that formate incorporation into uterine tissue is increased by the addition of *o*-hydroxylated derivatives of œstradiol *in vitro*. Finally, 2-methoxyœstrone[5,6] and 2-methoxyœstriol[7] have been isolated from human urine. It is therefore of importance to devise practical syntheses of the 2-hydroxyœstrogens, in order to facilitate further biochemical and biological investigations.

Mueller has outlined a procedure for the preparation of 2- and 4-hydroxyœstradiol via 2- and 4-nitroœstrone, but has given no experimental details or physical constants for the intermediate or final compounds. Recently the partial synthesis of 2-hydroxyœstradiol, starting from 19-nortestosterone-17β acetate, has been described by Axelrod and Rao[8]. A method for *o*-hydroxylation of phenols due to Loudon and Scott[9] has been employed by Fishman[10] to convert œstradiol-17β into 2-methoxyœstradiol-17β and 2-methoxyœstrone. This method has now been adapted to the partial synthesis of 2-hydroxyœstradiol 17β and 2-hydroxyœstrone from œstradiol-17β, and to 2-hydroxyœstriol from œstriol.

Œstradiol-17β was converted into 2-hydroxy-17β-acetoxyœstradiol-3-(2-benzoyl-4-nitrophenyl) ether by Fishman's method, and this ether was cleaved with boiling piperidine. The reaction mixture, after separation by chromatography on silica gel, afforded 2-hydroxyœstradiol-17β acetate, m.p. 181°. Hydrolysis with cold *N* sodium hydroxide in nitrogen gave 2-hydroxyœstradiol - 17β, m.p. 161–162°, $\lambda_{max.}^{EtOH}$ 289 mμ (ϵ, 4,187), $\lambda_{min.}^{EtOH}$ 254 mμ (ϵ, 543), $[\alpha]_D^{19°}$ + 89° (ethanol), $\nu_{max.}^{Nujol}$ 3,350 (broad), 1,600, 1,510 cm^{-1}. (Found: C, 75·15; H, 8·3. Calculated for $C_{18}H_{24}O_3$: C, 75·0; H, 8·4 per cent.)

Treatment of 2-hydroxyœstradiol-17β with exactly 2 moles of acetic anhydride in dilute pyridine solution at 0° gave predominantly the 2,3-diacetate, m.p. 106–107°, isolated by chromatography on alumina. The infra-red spectrum showed a single, intense, ester band at 1,760 cm.$^{-1}$. Chromium trioxide in acetone-sulphuric acid smoothly oxidized this secondary alcohol to 2,3-diacetoxyœstrone, m.p. 170–171°. Hydrolysis as before yielded 2-hydroxyœstrone, m.p. 193–194°, $\lambda_{max.}^{EtOH}$ 289 mμ (ϵ, 4,296), $\lambda_{min.}^{EtOH}$ 255 mμ (ϵ, 527), $[\alpha]_D^{19°}$ + 160° (ethanol), $\nu_{max.}^{CHCl_3}$ 3,550 (sharp) and 3,250 (broad), 1,635, 1,600 cm.$^{-1}$. (Found: C, 75·55; H, 7·9. Calculated for $C_{28}H_{32}O_3$: C, 75·5; H, 7·75 per cent.)

Œstriol was condensed with 2-chloro-5-nitrobenzophenone to give the 3-(2-benzoyl-4-nitrophenyl) ether, m.p. 130°, which yielded the 16,17-diacetate, m.p. 84–85°, with acetic anhydride in boiling pyridine. Cyclization of the diacetate with cold, concentrated sulphuric acid, followed by oxidation with hydrogen peroxide in acetic acid, gave the 2-hydroxy-3-ether. The latter was cleaved with boiling piperidine, and after chromatography on silica gel, 2-hydroxyœstriol-16,17-diacetate, m.p. 176–178°, was isolated. Alkaline hydrolysis, in the absence of oxygen, yielded 2-hydroxyœstriol, m.p. 267° (sealed, evacuated capillary tube), $\lambda_{max.}^{EtOH}$ 289 mμ (ϵ, 3,656), $\lambda_{min.}^{EtOH}$ 254·5 mμ (ϵ, 562), $[\alpha]_D^{19°}$ + 44° (ethanol), $\nu_{max.}^{Nujol}$ 3,420 and 3,190 (broad band), 1,600, 1,500 cm.$^{-1}$.

All the above intermediates for which a melting point is quoted were obtained in the pure crystalline form and show satisfactory analytical figures. The yields were generally good, and these routes therefore provide practical partial syntheses of these suspected metabolites from the available natural hormones.

The 2-hydroxyœstrogens are colourless, crystalline substances which dissolve freely in aqueous alkali, and may be recovered unchanged from their colourless solutions provided oxygen is excluded. In air or oxygen, rapid autoxidation occurs; approximately five atoms of oxygen per molecule of œstrogen are absorbed in a few minutes, with the production of an orange coloration, and the destruction of the aromatic *A* ring. Strong reducing properties are demonstrated by the ability of these compounds, as spots on paper, to give a blue coloration with the Folin-Ciocalteu reagent before exposure to ammonia vapour. The R_F values for paper chromatograms run with the system acetic acid (70) – water (30) – methylenedichloride are: 2-hydroxyœstrone, 0·73; 2-hydroxyœstradiol-17β, 0·59; 2-hydroxyœstriol, 0·23 (œstradiol-17β, 0·78; œstriol, 0·54).

Further work on the chemistry of these compounds is in progress, and the results of this, together with details of the syntheses outlined above, will be reported elsewhere.

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Lysosomal-like Particles in the Rat Mammary Gland

De Duve *et al.*[1] have described the occurrence in rat liver of a new type of cytoplasmic particle, the lysosome, which was characterized by the following properties: (1) A distinct enzymic composition, containing a number of hydrolytic enzymes (acid phosphatase, β-glucuronidase, acid ribonuclease and acid deoxyribonuclease). (2) They can be isolated as a distinct intermediate fraction, between the mitochondria and microsomes, by differential centrifugation. (3) When isolated undamaged, the lysosomes show a low level of enzyme activity, but when the membrane is ruptured by freezing, and thawing, or by the addition of non-ionic detergents such as 'Triton-*X*-100', there is a considerable increase in the activity of the enzymes.

Recently, several other hydrolytic enzymes (aryl sulphatase, phosphoprotein phosphatase and β-glucosidases) have been reported to show properties similar to those outlined above (for references see ref. 1), and lysosomal-like particles have been reported to occur in a variety of tissues including rat brain, mouse liver, kidney and *S*.37 tumour[2].

We have investigated the possibility that similar particles exist in rat mammary gland by studying the effects of freezing and thawing and of 'Triton-*X*-100' on enzyme activation, and by comparing the sedimentation properties of these enzymes with mitochondrial and microsomal components.

The rats used were adult females weighing about 200 gm. The litters of lactating mothers were restricted to 6–10 pups. Animals were killed by cervical dislocation, and the abdominal and upper inguinal glands were quickly dissected and placed into ice-cold aqueous 0·25 *M* sucrose. The tissue was homogenized in an Emanuel and Chaikoff homogenizer[3]. The tissue was first forced through the homogenizer using a clearance of 64μ and the intractable connective tissue was removed by straining through cheese-cloth. The suspension was then finally forced through using the rod giving a clearance of 17μ. The whole operation was done in a cold room at 4°. Enzyme assays on such suspensions were carried out as follows: cathepsin by the method of Greenbaum and Greenwood[4] using hæmoglobin as substrate; phosphoprotein phosphatase by the method of Paigen[5]; acid ribonuclease as described by Slater[6]; β-glucuronidase as outlined by Greenbaum and Greenwood[4]; acid deoxyribonuclease by the method of de Duve *et al.*[7] and acid phosphatase by the method of Berthet and de Duve[8]. The activities found for both phosphoprotein phosphatase and acid phosphatase during lactation were very low and no activation studies could be performed with these enzymes. Mammary gland acid deoxyribonuclease, although showing activation on the addition of high final concentrations of 'Triton-*X*-100', appears to differ from that of liver in that it did not seem to be associated with particles in mammary suspensions; little of the activity could be sedimented by centrifuging for 16,000*g* for 20 min. Other work suggests that this enzyme is largely found in the milk retained in the ducts and alveoli of the gland[9].

Cathepsin, acid ribonuclease and β-glucuronidase were, however, present in amounts suitable for activation studies. It was found that the activities of β-glucuronidase and acid ribonuclease were greatly increased by freezing and thawing the mammary suspensions or by the addition of 'Triton-*X*-100' as described by de Duve *et al.*[10]. Experimental results are shown in Fig. 1. Cathepsin, however, showed only a slight activation on the addition of 'Triton-*X*-100'. The activity in particle preparations from lactating

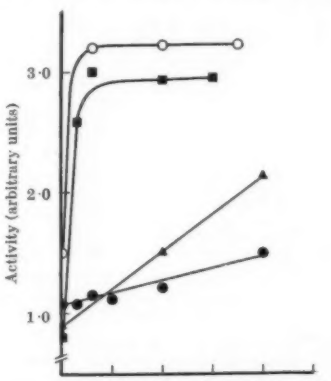

Fig. 1. Activation of acid ribonuclease (■), acid deoxyribonuclease (▲), cathepsin (●) and β-glucuronidase (○) by the addition of 'Triton-*X*-100' to the incubating medium. Enzyme activities are in arbitrary units and the procedures used for the assays are given in the text. All incubations were at 37° using mammary suspensions from rats in late lactation: the suspensions were prepared in 0·25 *M* sucrose using an Emanuel–Chaikoff type homogenizer[3]. The incubation times were 10 min. for acid ribonuclease and acid deoxyribonuclease, 20 min. for β-glucuronidase and 15 min. for cathepsin. The incubation medium was adjusted to a final concentration of 0·25 *M* sucrose with 1·7 *M* sucrose.

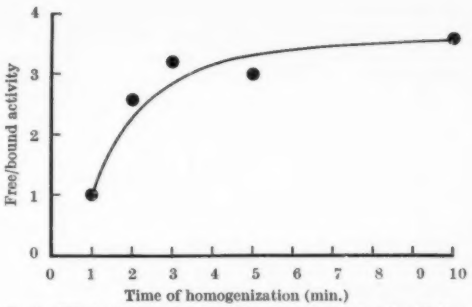

Fig. 2. Effect of increasing the time of homogenizing in a Potter–Elvehjem type homogenizer on the ratio of free/bound acid ribonuclease activity in rat mammary tissue suspensions taken from an animal in late lactation. The assay procedure for acid ribonuclease was as described by Slater[6] and consisted of 1 ml. of ribonucleic acid in acetate buffer (0·1 *M*, *p*H 5·8, 4 mgm. ribonucleic acid per ml. buffer) + 0·2 ml. tissue suspension + 0·3 ml. 1·7 *M* sucrose. For total ribonuclease activity 0·1 ml. 1 per cent 'Triton-*X*-100' was added to the incubation mixture. The difference total activity minus free activity is the bound activity, that is, the activation produced by 'Triton-*X*-100'.

mammary gland suspensions was generally increased by about 30 per cent on the addition of 'Triton-*X*-100' in a final concentration of 0·2 per cent. This behaviour is analogous to the results of Beaufay and de Duve[1] on liver cathepsin.

When tissue suspensions of mammary gland in 0·25 *M* sucrose were centrifuged at 16,000*g* for 20 min. at 0° in an Angle 13 M.S.E. centrifuge, 70–80 per cent of the total activity of acid ribonuclease, β-glucuronidase and cathepsin was sedimented. On the other hand, if homogenization was carried out using a conventional Potter–Elvehjem type homogenizer with a plastic pestle (clearance 0·01 in.) for 2 min., more than 70 per cent of the total enzyme activity was in a non-sedimentable form and little activation by 'Triton-*X*-100' could be demonstrated. Thus it appears that homogenizing of mammary tissue with the Potter–Elvehjem technique causes damage to the particles containing these three enzymes; the damage caused was proportional to the time of homogenizing at 0° as shown in Fig. 2 for acid ribonuclease. Times of homogenizing up to 2 min. in the Potter–Elvehjem instrument, however, had no detectable influence on the sedimentation properties of mitochondrial enzymes cytochrome oxidase and succinate neotetrazolium reductase. Breakage of the particles containing cathepsin, β-glucuronidase and acid ribonuclease, with consequent lack of further activation by 'Triton-*X*-100', was also achieved by high-speed blending, and by incubating in hypotonic sucrose solutions.

It would appear that acid ribonuclease and β-glucuronidase behave similarly in mammary suspensions in response to osmotic activation, to freezing and thawing and to the addition of 'Triton-*X*-100'; on centrifuging in 0·25 *M* sucrose the two enzymes sediment in the same fraction as cathepsin. Their behaviour therefore closely parallels the properties reported by de Duve *et al.*[7] for liver lysosomes. One point of great interest arising out of this work on mammary tissue suspensions concerns the variation in total enzyme activities over the lactation-cycle. Fig. 3 shows the variations in the total activities of cathepsin[4], β-glucuronidase[4] and acid ribonuclease[6] as reported previously. It can be seen that these three enzymes, which show very similar properties with respect to activation and centrifuging, have very different patterns of change over the lactation-cycle. It would seem that if these three enzymes are located

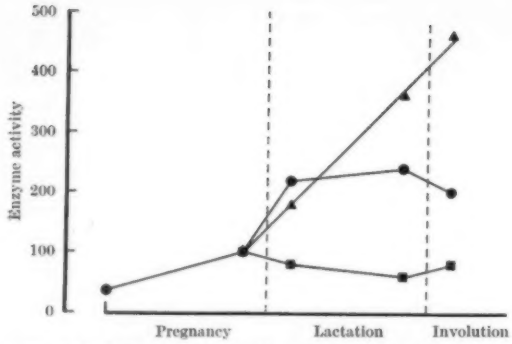

Fig. 3. Variation in β-glucuronidase (■), acid ribonuclease (●) and cathepsin (▲) in rat mammary gland suspensions over the lactation cycle. Figures taken from the literature: for β-glucuronidase and cathepsin from Greenbaum and Greenwood[4], for acid ribonuclease from Slater[6]

within a similar type of lysosomal particle in rat mammary tissue, either independent variations in enzymic activities can occur at different physiological states or there is a variety of lysosomal particles each with specific enzymic constitutions. This latter possibility has been mentioned by Conchie and Levvy[2] and by Beaufay and de Duve[1].

It seems of interest to follow the ratio of bound/free lysosomal enzyme activity over the lactation cycle particularly in mammary involution where the tissue is degenerating rapidly. This is under investigation.

This work was done during the tenure of a Beit Memorial Fellowship held by one of us (T. F. S.) and of a Medical Research Council Studentship held by another (D. Y. W.).

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Base Composition of Ribonucleic Acid from Rat Mammary Gland during Pregnancy and Late Lactation

Ribonucleic acid has become increasingly associated with pathways of protein biosynthesis in many different types of tissue and there is a widely held belief that the sequential order of nucleotide bases in a particular ribonucleic acid molecule governs the amino-acid order of protein synthesized in its vicinity[1]. Incorporation of amino-acid, which has been equated with protein synthesis under certain conditions, has been shown to occur under these conditions in the intracellular fractions from many tissues. Differences in the ribonucleic acid base composition of mitochondrial, microsomal and supernatant fractions have been found for rat liver[2,3].

The rat mammary gland undergoes a rapid change in physiological activity at parturition. The rate of protein synthesis increases rapidly, and concomitant with this there is a rapid increase in the gland's content of ribonucleic acid; this increase is largely centred in the particulate fractions of mammary suspensions[4].

We have investigated the possibility that the sudden increase in both ribonucleic acid and protein synthesis occurring over parturition is accompanied by an alteration in the base composition of the newly synthesized ribonucleic acid. It was thought possible that the increase in the synthetic rate of the specific milk protein, casein, occurring over parturition might be sufficiently drastic to produce detectable alterations in the overall ribonucleic acid base composition. It was also of interest to study the base composition of lactating mammary gland ribonucleic acid to compare the acid-base composition of such an active protein secretory tissue with other tissues previously investigated.

The rats used were primiparous adult females the litters of which were restricted to 6–10 pups. All animals in the early pregnancy group were between 14–15 days pregnant; animals in the late-pregnancy groups were 17–20 days pregnant; the late-lactating groups were made up from animals 17–19 days lactating. Animals were killed by cervical dislocation and the abdominal and inguinal glands were quickly dissected and placed into ice-cold water. Tissue homogenates were prepared using a M.S.E. top-drive blendor and the cold suspension strained through muslin to remove intractable connective tissue. For the experiments involving separations of intracellular fractions, homogenates were prepared in ice-cold 0·25 *M* sucrose using an Emanuel–Chaikoff homogenizer[5]; cell debris and nuclei were removed by preliminary centrifuging at 600*g* for 10 min. and the supernatant from this was then centrifuged at 105,000*g* for 20 min. at 0°. The sediment was resuspended in water to give the original concentration.

Ribonucleic acid was extracted from homogenates or tissue fractions essentially by the phenol method of Kirby[6]. The nucleotide base composition of ribonucleic acid samples was determined by the method of Herbert, Potter and Hecht[7] using 'Dowex-1' resin in the formate form. The column size was 5 cm. × 0·72 cm.^{2}; the rate of flow was approximately two 15 ml. fractions per hour.

The results for the base composition of mammary gland ribonucleic acid at three stages of the lactation-cycle are shown in Table 1. It can be seen that there is no significant alteration in the ratio of purine to pyrimidine bases at the three stages of the lactation-cycle studied. Thus, although the mammary gland changes very rapidly in protein synthetic ability during lactation, no significant alterations in the gross base composition of whole homogenate ribonucleic acid could be detected. Whereas in late pregnancy the protein synthetic machinery of the gland is concerned largely with the maintenance of the tissue protein itself, in lactation the emphasis is largely on the production of a very different protein, casein. Assuming that a close inter-relationship exists between ribonucleic acid and protein synthesis, it may be concluded that if differences in ribonucleic acid occur concomitant with a change over to the synthesis of a new protein, the differences are subtle and are not detectable by the type of overall base composition

Table 1. NUCLEOTIDE BASE COMPOSITION OF RIBONUCLEIC ACID SAMPLES EXTRACTED FROM MAMMARY GLAND TISSUE SUSPENSIONS AT THREE STAGES OF THE LACTATION CYCLE

The results are given as moles of nucleotide per 100 moles ribonucleic acid. A 12 per cent correction has been applied for the deamination of cytidylic to urid ylic acid

Stage of lactation cycle	No. of animals	CMP†	AMP	UMP	GMP
Early pregnancy	4	28	20	16	36
	4	27	22	15	36
Mean		27·5	21	15·5	36
Late pregnancy	3	29	21	14	36
	3	28	20	14	37
	2	29	20	14	37
	2	26	20	18	35
Mean*		28 ± 0·7	20 ± 0·3	15 ± 1·0	36 ± 0·5
Late lactation	1	27	16	17	40
	1	26	21	16	38
	1	28	21	15	36
	1	27	22	15	35
	1	29	15	16	37
	1	27	19	17	37
	1	27	22	15	37
	1	27	18	15	40
Mean*		27 ± 0·3	19 ± 0·8	16 ± 0·3	37 ± 0·7

* ± *S.E.M.*
† Abbreviations: CMP, cytidine monophosphate; AMP, adenosine monophosphate; UMP, uridine monophosphate; GMP, guanosine monophosphate.

determinations reported in Table 1 for whole homogenate ribonucleic acid.

The base composition of mammary gland ribonucleic acid appears to be somewhat different from the base composition of rat liver acid. Rat liver (and rat kidney) ribonucleic acid has been shown to have a purine/pyrimidine ratio of unity[2,8,9]. Rat mammary gland acid, however, has a ratio of 1·34 but the ratio of 6-keto groups to 6-amino groups is 1·10 which is close to the corresponding value (1·08) given by Elson and Chargaff[8] for rat liver ribonucleic acid. It would thus appear that rat mammary gland ribonucleic acid has approximately equal numbers of 6-keto and 6-amino groups but that the added regularity of a purine/pyrimidine ratio of unity is not present.

The results for the base composition of ribonucleic acid from intracellular fractions of rat mammary tissue taken from animals in late lactation are shown in Table 2. A major role in protein biosynthesis has been ascribed to the microsomal fraction of many tissues. However, it can be seen from Table 2 that in late lactation, when casein synthesis is at its peak, the particulate fraction of mammary tissue suspensions shows no significant differences in ribonucleic acid base composition from the soluble fraction remaining after approximately 3×10^6 g.min. There are indications that the nuclear fraction from mammary suspensions has a lower purine/pyrimidine ratio than the other fractions; a similar low ratio for liver nuclei has been previously reported[2].

Table 2. BASE COMPOSITION OF RIBONUCLEIC ACID SAMPLES EXTRACTED FROM INTRACELLULAR FRACTIONS OF RAT MAMMARY GLAND SUSPENSIONS TAKEN FROM ANIMALS IN LATE LACTATION

For details of the centrifugings see the text. The results are expressed as percentages of the corresponding whole homogenate value for each base ratio (that is, the whole homogenate value for all ratios is 100)

Fraction	No. of experiments*	Molar ratios compared to whole homogenate value of 100 AMP/CMP	UMP/CMP	GMP/CMP	Purine/pyrimidine compared to whole homogenate value of 100
Nuclear	2	93	173	113	77
Particles	4	100±5	87±5	97±4	105±3
Supernatant	6	116±14	100±7	105±7	110±8

* Two animals used per experiment.

Total ribonucleic acid estimations were performed on the intracellular fractions, and the results were in agreement with earlier work on the distribution of ribonucleic acid in mammary tissue[4]. The bulk of the homogenate ribonucleic acid is associated with the particulate fraction (70–80 per cent); only some 10–20 per cent of the total suspension ribonucleic acid was found in the soluble fraction with the centrifuging procedure used. Thus the purine/pyrimidine ratio of the whole homogenate ribonucleic acid is largely determined by the purine/pyrimidine ratio of the particulate fraction. This is shown in Table 2, where it can be seen that the purine/pyrimidine ratio for the particulate fraction is not statistically different from the whole homogenate value.

Ribonuclease did not have any appreciable effect on the bulk of the ribonucleic acid since in experiments where homogenates were allowed to stand for 2 hr. at 0° no differences in the purine/pyrimidine ratios were observed compared with similar homogenates placed in phenol immediately after homogenization (2 hr. was approximately the time taken to prepare the intracellular fractions before phenol extraction). It has been shown that ribonuclease is associated with lysosomal-like particles in rat mammary tissue[10] and that when a mammary suspension is prepared using an Emanuel–Chaikoff type homogenizer, only some 10 per cent of the total ribonuclease is in a free and non-sedimentable form.

It would thus appear from the results obtained in this investigation that there is no gross alteration in the base composition of mammary gland ribonucleic acid at the three stages of the lactation-cycle studied. Further, during late lactation, there are only small differences between the base compositions of ribonucleic acid extracted from intracellular fractions. Since the differences in composition of ribonucleic acid, which almost certainly occur in the mammary gland in response to the stress of lactation, cannot be detected by overall base composition analyses a study has been initiated on the composition of ribonucleic acid species isolated by chromatographic techniques similar to those used in the studies of Goldthwaite[11] on liver.

This work was done during the tenure of a Beit Memorial Fellowship held by one of us (T. F. S.) and of a Medical Research Council Studentship held by D. Y. W.

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Binding of Zinc in Carboxypeptidase

Carboxypeptidase is a metallo-enzyme which contains zinc. It has been reported by Coleman and Vallee[1] that the enzyme can be de-activated by removal of zinc and that activity can be restored by the addition of zinc, cobaltous, ferrous, nickel and manganous ions but not by addition of cadmium, magnesium or calcium ions. This metallo-enzyme therefore falls in the group of enzymes which have no specific metal requirement for activity[2]. In biological systems, however, a particular metal, zinc, is associated with the enzyme presumably because complex ion equilibria favour this particular association over all others. The situation is not very different from that found in the case of the enzyme enolase, where magnesium and manganous are the particular ions associated with activity in the biological system, but where other cations such as zinc can activate the enzyme *in vitro*[3]. The apparent stability constants for the association of zinc and cobaltous ions with carboxypeptidase are 2×10^8 and 1×10^6 respectively[1]. The *p*H range of activation by the different cations indicates that association with the active centre is in the order zinc(II) > nickel(II) and cobalt (II). The study of complexes of a wide variety of simple organic ligands has shown[2] that this stability sequence is observed only when one of the co-ordinating centres is sulphide, *R*—S⁻. I conclude that zinc is bound by a sulphide group in carboxypeptidase and give the following additional evidence in support of this binding.

The cobaltous carboxypeptidase complex is highly coloured. The molar extinction coefficient at 530 mμ is 150[1]. The usual value of the extinction coefficient of cobaltous complexes is < 10, except when the cobalt ion is bound to large, polarizable ligand atoms such as chloride and sulphide[4]. Thus both cobaltous thiocyanate and thiosulphate have extinction coefficients of the order of 10^2, and cobaltous cysteine and thioglycollate complexes are highly coloured. In the complex with carboxypeptidase, the binding of the cobaltous ion is probably restricted to oxygen, nitrogen or sulphur centres, and it would appear that only in the case of binding to sulphur is the colour of the complex explicable from the known properties of model complexes. The shift of the absorption band from 512 mμ in the hydrate to 530 mμ in the carboxypeptidase complex, that is, a shift to longer wavelengths, is in the expected direction if binding to cobalt is through a mixture of oxygen and sulphur co-ordinating centres, but is in the opposite sense from the shift produced by extensive co-ordination to nitrogen.

In the metallo-enzyme enolase I have postulated that the binding of the cations is largely through oxygen atoms[2]. In this enzyme magnesium is active. Magnesium cannot activate carboxypeptidase. It is not known to bind to sulphide groups. On the other hand, cations such as lead(II), cadmium, mercuric, silver, and cupric which bind sulphur groups more strongly than zinc may be expected to be powerful inhibitors.

If cobalt is bound to sulphur in carboxypeptidase, it is possible to predict that there will be a further new absorption band absent in the complexes of the other cation complexes of this enzyme. The new band will be at about 285–300 mμ and will have an extinction coefficient greater than 10^3. Both this strong band and the band in the visible of extinction coefficient 10^2 are present in model cobalt complexes containing Co—S—*R* links[4]. Neither of these bands is observed in cobaltous insulin, and we can predict that in this complex the binding of zinc is to a mixture of oxygen and nitrogen groups. Both insulin and carboxypeptidase contain sulphur groups.

Note added in proof. Since this communication was submitted I have heard from B. L. Vallee and his associates that they have demonstrated Zn—S binding independently.

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A Plate Assay for Elastase

Since Balo and Banga[1] described the pancreatic enzyme elastase, its dissolving effect on elastin, and its possible implications in atherosclerosis, a considerable amount of interest has been focused on the quantitative measurement of its enzymatic activity.

Two methods most commonly used for the assay of elastase are based on gravimetric measurements and nephelometry. Much has been learned in regard to the interaction of elastin and elastase and its inhibitor, serum, by these two methods. However, both methods suffer from the fact that they require much time and special equipment and skilled personnel.

If a programme designed to establish and follow elastase-inhibitor levels in sera from a variety of sources, including human sera, is anticipated, a relatively easy and rapid assay for elastase and its inhibitor is essential. Such a programme is in progress in this laboratory, and an assay fulfilling these requirements has been developed.

Table 1. Water–Sodium Hydroxide Elastin is Used. It is Autoclaved for 10 min. at 10 lb. Pressure. The Resulting *p*H is 7

Components	Composition of elastin medium: Amount of components in stock solution	Volume of stock solutions for 100 ml. of complete medium
Inorganic salts		
K_2HPO_4	25 gm.	
KH_2PO_4	25 gm.	
Distilled water	250 ml.	0·5 ml.
Inorganic salts *B*		
$MgSO_4\,7H_2O$	10 gm.	
NaCl, $FeSO_4\,7H_2O$	0·5 gm.	
$MnSO_4\,4H_2O$	of each	
Distilled water	250 ml.	0·5 ml.
Elastin	10 gm.	
Distilled water	100 ml.	10 ml.
Distilled water		87·5 ml.
Agar		1·5 gm.

Table 1 shows the composition of elastin agar. 20 ml. of this medium is poured into a sterile 15 × 150 mm. Petri dish. The non-transparent medium (due to the presence of elastin) is allowed to harden. Six wells are made in the medium by either allowing the medium to harden in the presence of plastic molds which can then be easily removed after hardening, or the medium is allowed to harden and the molds are pushed through the agar. The resulting cylinder of agar can be teased away with an inoculating needle. The wells serve as reservoirs for elastase and its inhibitor serum. The diffusion of elastase from the wells and its subsequent dissolution of elastin result in clear zones around the wells, the zones varying in diameter with the concentration of elastase used. The resulting clear zones can be seen

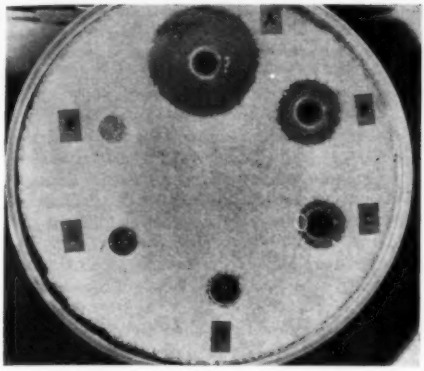

Fig. 1. Photograph of an elastin agar plate showing solubilization (clear zone) of elastin around the wells containing decreasing concentration of elastase, wells *A*–*E* (5, 4, 3, 1, 0·5 mgm.); *F* is a buffer control. 0·06 ml. of bovine pancreatic elastase in the desired concentration is placed in each well. An equal amount of buffer (not containing elastin) is placed in well *F*. The plates are allowed to incubate right side up for 72 hr. at room temperature. The zone of solubilization can be read at each 24-hr. interval. Bovine pancreatic elastase was prepared according to the method of Lewis (ref. 3). Water–sodium hydroxide elastin was prepared by the method described by Gilfillan *et al.* (ref. 4)

in Fig. 1. Inhibition of elastase can be easily demonstrated by the addition of serum to the reaction well. The results obtained when a constant amount of elastase is placed in each of five different wells along with decreasing concentrations of serum are illustrated in Fig. 2. The inhibitory effect of serum is obvious. Partridge and Davis[2] have shown that elastin degradation is accompanied by the liberation of N-terminal substances. Ninhydrin added to the well surrounded by the clear area shows a bluish-purple area indicating the liberation of free amino groups.

Preliminary work indicates that this plate assay correlates well with the nephelometric–ninhydrin method used by Gilfillan *et al.*[4].

We believe that the elastase plate assay described is less time-consuming, requires less special equipment, does not require skilled personnel, and most important is readily adaptable to assaying large numbers of sera for elastase inhibitor or tissue, bacterial, and fungal extracts for elastase.

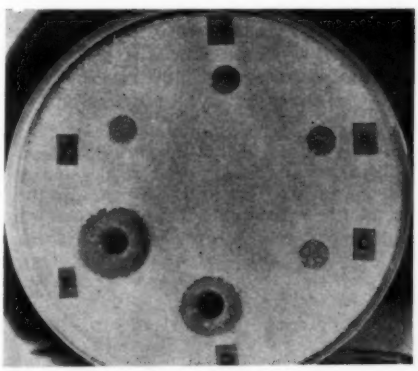

Fig. 2. Photograph of an elastin agar plate showing the inhibitory effect of third trimester pregnancy serum on elastase. The elastase concentration (5 mgm./well) was constant in wells *A*–*E*. Serum concentrations decreased from *A* to *E* (1/20, 1/40, 1/60, 1/80, 1/160). Well *F* contained an equal amount of serum and served as a control. Plates are incubated and read as in Fig. 1. (Photographs taken by George Daynes)

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PHYSIOLOGY

Specific Activities of Acid-soluble Nucleotides in Hind-Leg Muscles of Mice with Dystrophia Muscularis

In an earlier paper[1], we showed that the concentrations of adenosine monophosphate, guanosine triphosphate and guanosine diphosphate were significantly higher in the hind limb muscles of dystrophic mice than those obtained for normal mouse muscles, while for adenosine triphosphate the reverse was found. Although the concentration of the adenosine diphosphate component was the same for both normal and dystrophic tissue, radioautographs of nucleotides containing radioactive phosphorus seemed to show an area of higher radioactivity for the adenosine diphosphate derived from the dystrophic muscle. The present work was designed to examine the turn-over rates of the acid-soluble nucleotides in the skeletal muscle of normal mice and mice afflicted with a hereditary myopathy (dystrophia muscularis).

Both dystrophic mice and their normal litter mates (obtained from the Roscoe B. Jackson Memorial Laboratories, Maine) were injected subcutaneously with carrier-free radioactive phosphorus (20 μc./gm. body-weight). Two hours later, the animals were anæsthetized and the hind leg musculature was excised. In order to minimize the enzymatic degradation of the nucleotides, the muscle was handled at temperatures of 0° C. or below[1].

After weighing the frozen tissue on a torsion balance, the muscle was transferred to a homogenizing flask containing 10 ml. of 0·25 *M* perchloric acid (1–2° C.) per gm. of tissue[2]. 0·5–1·0 gm. of muscle tissue was available for each analysis. The sample was homogenized for 10–20 sec. at 40,000 r.p.m. and then centrifuged (3,000 r.p.m.) for 15 min. at 0° C. A 10 per cent suspension of acid-washed 'Norite' (0·4 ml./gm. of extracted muscle tissue) was added to the supernatant and the emulsion was agitated for 30 min. in the cold[3–5]. The 'Norite' was separated by centrifugation, washed with water and recentrifuged. 10 per cent pyridine in 60 per cent ethanol (8 ml./gm. extracted muscle tissue) was added to the 'Norite'. The contents of the tubes were mixed, and elution was allowed to proceed over a 2-hr. period at 37° C. with occasional mixing[5]. The charcoal was removed by centrifugation, and the eluate containing the acid-soluble nucleotides was measured. Aliquots were taken for the measurement of radioactivity present in the total extracted nucleotides, and the remaining eluate was concentrated to a small volume.

The concentrated nucleotides were quantitatively transferred to Whatman No. 1 filter paper and re-

Table 1. Specific Activities* of Acid-soluble Nucleotides found in Muscular Tissue (Hind Legs) of Normal and Dystrophic Mice†

Cases	Adenosine monophosphate	Adenosine diphosphate	Adenosine triphosphate	Diphosphopyridine nucleotide	Guanosine triphosphate‡	Guanosine diphosphate‡
4 Normal	67,900	149,400	477,700	12,000	408,800	310,000
S.D.	±15,400	±26,200	±88,800	±2,200	±84,600	±62,000
5 Dystrophic	50,900	226,700	552,300	24,500	493,400	478,600
S.D.	±9,100	±24,500	±39,800	±4,900	±45,300	±32,300
P	<0·05	<0·001	<0·05	<0·001	0·05	<0·001

* Specific activity = $\frac{\text{counts per min.}}{\mu\text{mol. of nucleotide (average of 18 determinations)}}$

† Animals killed 2 hr. after the subcutaneous injection of radioactive phosphorus.

‡ Guanosine tri- and di-phosphate contain other nucleotides (ref. 1).

solved by descending chromatography for 40 hr. using the *iso*butyric acid–ammonium *iso*butyrate solvent system of Magasanik[6]. Following resolution of the unknown mixture, 6 spots corresponding to adenosine monophosphate, diphosphopyridine nucleotide, adenosine diphosphate, adenosine triphosphate, guanosine di- and tri-phosphate were made visible with ultra-violet light. However, radioautographs prepared from the chromatogram indicated the presence of an unidentified radioactive phosphorus-containing substance in the adenosine monophosphate–diphosphopyridine nucleotide area, which did not absorb ultra-violet light. Since we were interested in the specific activity of each component, and this non-nucleotide substance might contribute to the specific activity of either adenosine monophosphate or diphosphopyridine nucleotide, it was necessary to remove this compound before eluting the adenosine monophosphate and diphosphopyridine nucleotide. This was accomplished by ascending chromatography in the second dimension for 5 hr. at 20° C. using the *n*-butanol/*n*-propanol/formic acid (10 : 40 : 30) solvent of Venkstern and Baev[7]. With this solvent, the unidentified component as well as orthophosphate were resolved away from the nucleotides, which remained at the origin.

Quantitative elution and estimation techniques for the separated nucleotides were the same as described in the previous publication[1]. After determining the optical densities of the various eluates, aliquots were pipetted into metal planchets. The counting samples were dried and radioactivity measurements were determined with a Nuclear Chicago gas-flow counter.

The specific activities calculated for the acid-soluble nucleotides obtained from the hind-leg musculature of 5 dystrophic and 4 normal mice run by the above procedure are tabulated in Table 1. Significantly higher specific activities were found for the adenosine diphosphate (226,700), diphosphopyridine nucleotide (24,500), and guanosine diphosphate (478,600), from the dystrophic mouse muscle, as compared to normal values of 149,400 for adenosine diphosphate, 12,100 for diphosphopyridine nucleotide, and 310,000 for guanosine diphosphate. On the other hand, little or no differences existed in the specific activities of the adenosine mono- and tri-phosphate, and guanosine triphosphate derived from the two types of tissue.

Although the concentration of adenosine triphosphate from the dystrophic tissue is 30–40 per cent lower than that found in normal tissue, the specific activities for adenosine triphosphate from both types of tissue are not significantly different. While it has been postulated that adenosine triphosphate plays a significant part in all phases of muscle metabolism, the roles of the other acid-soluble nucleotides cannot be dismissed[8]. In view of the significantly higher specific activities of adenosine diphosphate, guanosine diphosphate and diphosphopyridine nucleotide, it is possible that they contribute through secondary pathways to make up for the deficiency in adenosine triphosphate.

A more comprehensive study of the turn-over rates of the various acid-soluble nucleotides is in progress.

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Storage and Release of Catecholamines, and the Occurrence of a Specific Submicroscopic Granulation in Hearts of Cyclostomes

The relatively large amounts of catecholamines found in the hearts of cyclostomes[1,2] suggest that these substances may be stored in specific cells or cellular structures. Preliminary examination with the light microscope of specimens from the hearts of the lamprey (*Petromyzon fluviatilis*) and the hagfish (*Myxine glutinosa*) indicated the presence of a specific cell type possibly serving as store. It was therefore considered of interest to analyse different cardiac structures of these two cyclostomes with regard to their content of catecholamines and to make a parallel study of the morphology of this unknown cell with the aid of the electron microscope.

Adrenaline and noradrenaline were determined fluorimetrically by the method of Euler and Lishajko[3] after extraction of the hearts with 5 per cent trichloracetic acid, adsorption on alumina and elution

with acetic acid. Column chromatography of an eluate obtained with sulphuric acid was used for the determination of catechol compounds in general. A granular fraction was also isolated by high-speed centrifugation of press juice from the hearts according to the method described by Euler[4], using 0·3 *M* and 0·075 *M* potassium phosphate as medium for *Myxine* and *Petromyzon* hearts, respectively.

For histological examination specimens were freeze-dried or fixed in various fixatives and were studied under light and fluorescence microscopes. For electron microscopy specimens were fixed in buffered osmic acid and embedded in methacrylate; sections were examined in an R.C.A. type *EMU 2 B* electron microscope.

Table 1. Adrenaline (A) and Noradrenaline (NA) in Hearts of *Petromyzon fluviatilis* (Lamprey) and *Myxine glutinosa* (Hagfish) in μgm per gm.

	Auricle		Ventricle		Portal heart	
	A	NA	A	NA	A	NA
Lamprey	140	3·4	25	0	—	—
	118	7·6	24	0	—	—
	132	7·9	35	0	—	—
Hagfish	15	40	40	5·6	3·1	53
	11	54	49	6·4	0·3	59
	—	—	—	—	4·5	44

Table 1 shows the amounts of adrenaline and noradrenaline in the auricle and the ventricle of lamprey and hagfish and also in the portal heart of hagfish. It can be seen that the lamprey heart contains chiefly adrenaline which is present in large amounts in the auricle as well as in the ventricles. In the hagfish a peculiar distribution of the two amines is noted in that the portal heart and the auricle contain mainly noradrenaline, while adrenaline is the dominating amine in the ventricle.

By column chromatography of heart extracts of hagfish and lamprey it could be shown that adrenaline and noradrenaline were the predominant catechol compounds and that dopamine and dihydroxyphenylacetic acid were present only in very small amounts. In fractions of isolated granules the proportion of the two amines was approximately the same as that found in total extracts.

It has recently been shown that reserpine causes a release of noradrenaline from isolated transmitter granules from mammalian splenic nerves[5]. Incubation of isolated granules from *Petromyzon* and *Myxine* hearts with reserpine (0·1 mgm./ml. for 30 min. at 20° C. and *p*H 6·1) increased the release of catecholamines from the granules by about 30 per cent as compared with the controls.

After 6 daily injections of 0·1–0·2 mgm. reserpine subcutaneously the amounts of adrenaline and noradrenaline in the hearts of *Myxine* and *Petromyzon* were reduced to 15–30 per cent of the control values.

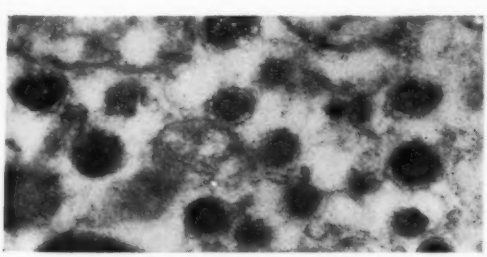

Fig. 1. Electron micrograph showing a detail of a specific granular cell from heart ventricle of *Myxine*

Electron micrographs of specimens of hearts from both species have revealed the occurrence of numerous cells containing large numbers of dense, osmiophilic granules, round in shape and measuring in diameter about 0·1μ. The granules generally show a distinct border surrounded by a lighter space which in turn is encircled by a limiting membranous structure (Fig. 1). With regard to the localization of these granules, this varies in a somewhat puzzling way. In the hagfish auricle, accumulations of granules have been observed in what appears to be ordinary heart muscle cells. However, in both species there appear to be present specific cells, extremely rich in granules of the type described. Hitherto two types of such cells have been observed, one characterized by a dark-staining cytoplasm which is completely packed with granules, and the other showing a light, almost empty-looking cytoplasm with only few granules. It is at present not possible to state if two different cell types really exist or if the varying appearance may be due to different functional stages of one and the same cell type.

The granular structures found in the hearts of these two fish species show a striking morphological resemblance to the granules found in cells of the adrenal medulla of the rat[6] and of the mouse, guinea pig and cat[7]. Furthermore, the varying appearance of the cells is very similar to that described by Lever[6] in adrenal medullary cells of the rat. He describes: "a dark cell in which abundant osmiophilic adrenaline granules are contained in a relatively opaque background cytoplasm and a light cell containing fewer secretory granules".

It is at present not possible to draw definite conclusions as to the true nature of the granular cells encountered in hearts of the cyclostomes *Myxine* and *Petromyzon*. However, taking into consideration the large amounts of catecholamines present in heart tissues of these fishes and the interesting observation that characteristic nervous elements seem to be lacking in *Myxine*[2] it seems possible that we are here dealing with a cell type belonging to the chromaffin system.

This investigation is being continued, and a full report will be presented elsewhere.

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Cannulation of Minute Mesenteric Veins for Continuous Portal Perfusion of Rats

The introduction of a cannula into a very small vein (< 1 mm.) is almost impossible because of collapse and cohesion of the vessel walls. Any looping or clamping of the vein beyond the incision in order to avoid bleeding and to maintain a clear visual field causes spasm which will not permit further passage of the tubing into the vascular lumen. If the cannula is forced, it will perforate the thin vessel. We have, therefore, constructed a cannulating device which permits the introduction of a fine plastic tubing into a small vessel without visual control.

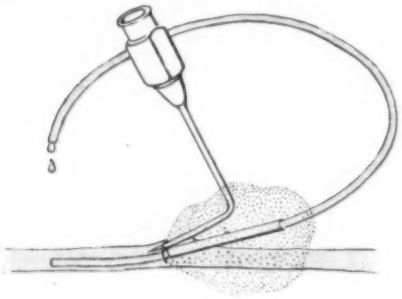

Fig. 1

The device consists of a No. 22 injection needle, 1½ in. long, the distal ½ in. of which is bent to a right angle so as to form a trough terminating at the needle point. To this trough, and just at the base of the needle point, is soldered a straight 1 in. long metal cannula, which forms an angle of about 15° with the base of the needle trough (Fig. 1). A polyethylene tubing (No. 10 Clay-Adams) with a square end is introduced into the metal cannula which will guide the plastic tubing exactly to where the point of the angled needle has punctured the vessel. When the plastic tubing is now pushed forward, it passes close to and beneath the needle point into the vascular lumen (Fig. 1). In spite of lack of visibility due to the bleeding from the punctured vessel, successful cannulation is evident from the flow of blood into the tubing. Threads which have been previously passed under the vessel and looped are tied after the point of the cannulating device has been pulled back about 3 mm. The plastic cannula is now firmly grasped with a thumb forceps in front of the needle point and held in position while the cannulating device is slipped off the tubing. The polyethylene tubing is filled with a 1:10,000 heparin–saline solution and stoppered. Eighty-eight female rats weighing 220–260 gm. were anæsthetized with intraperitoneal 'Nembutal' (6 mgm./100 gm. body-weight) and a laparotomy with exposure of the ileocæcum was carried out. The ileocæcal loop and its mesentery containing the exposed vein were spread on a thin cork plate covered with moist gauze. The mesentery was pinned down to the cork plate in order to permit the smooth sliding of the cannula into the stretched vein. The cannulating device containing the plastic tubing was attached to the barrel of a 2 c.c. syringe, thus providing a steady grip of the instrument. The cannulation of the ileocæcal vein by this technique is done close to the intestinal border of the mesentery. The abdomen is closed in two layers and the cannula brought out through the most cephalad part of the mid-line incision to prevent the rats from catching their hind-feet in the cannula. A small glass bead is slipped over and glued to the cannula at its point of exit from the abdominal wall. The cannula is fixed by putting a suture through the skin and tying it in front of the glass bead. The animals are then restrained[1] and continuously perfused with various solutions.

Of the 88 rats, 26 have been perfused for less than 3 days, 41 have been perfused for 4–10 days, and 21 have been perfused for more than 10 days.

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ANATOMY

Histochemical Demonstration of Aromatic Monoamine in the Locus Cœruleus of the Mammalian Brain

Shimizu *et al.* have reported several interesting histochemical results of the locus cœruleus of the mammalian brain—the strong activity of acid phosphatase[1] and cholinesterase[2] but rather poor action of respiratory enzymes[3,4], and further, characteristically strong monoamine oxidase activity[5]. The last finding led us to suppose that some aromatic monoamines such as catechol amines and serotonin would be concentrated in the locus. Although Falck and Hillarp[6] recently reported that no chromaffin reaction could be observed in the hypothalamus and the caudate nucleus, and concluded that noradrenalin and dopamine are not stored in a small number of nerve cells of these regions, they did not examine the locus cœruleus. In the present investigation several histochemical reactions including argentaffin, Schmorl's, chromaffin, azo-coupling and Gibbs's reaction were carried out in mammalian brain tissues containing the locus cœruleus.

The materials used were taken from the healthy adult rabbit, rat, mouse, guinea pig, dog and cat and also from the scorbutic guinea pig and animals treated with marsilid or catron. All tissue slices were fixed in 10 per cent neutral formalin or 5 per cent potassium bichromate–chromate solution with or without formalin[7]. The specimens were quickly embedded in paraffin, since a yellow compound due to an oxidation of chromaffin substances might be dissolved in hot paraffin[8]. The sections (7–10μ) were subjected to the following histochemical reactions: the argentaffin reaction (Gomori's methenamine silver or Fontana's ammoniacal silver solution, cf. Pearse[9]) for 24 hr. without pretreatment with Gram's iodine solution, Schmorl's ferricyanide reaction (Lillie and Burtner[10]), azo-coupling reaction (diazo blue *B* in alkaline solution, cf. Pearse[9]) and Gibbs's reaction (cf. Gomori[11]). The results are summarized in Table 1.

The silver or Prussian blue granules due to the presence of a reducing substance occurred almost exclusively in the locus cœruleus of all animals hitherto studied except for rats and mice, where the

Table 1. HISTOCHEMICAL REACTIONS OF THE REDUCING GRANULES OF THE LOCUS CŒRULEUS OF GUINEA PIGS

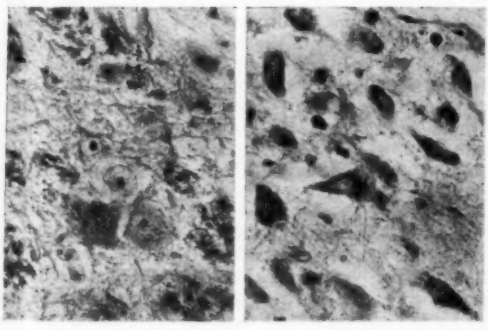
Fixation	Argentaffin reaction	Schmorl's reaction	Chromaffin reaction	Azo-coupling reaction	Gibbs's reaction
Formalin	++	++	−	±	−
Bichromate-formalin	+	±	−	−	−

−, No reaction; ±, slightly positive reaction; +, weak reaction; ++, moderate reaction.

granules were always recognized within the nerve cell bodies and their processes but not in the neurophil. It should be mentioned, however, that the granules did not occur equally in all specimens tested, and they were not always demonstrated in all nerve cell bodies of the nucleus, but their quantity and distribution varied according to the animal species used. The granules, for example, were observed most abundantly in the dog, cat and guinea pig (Fig. 1*a*) but slightly in the rabbit and were absent in the rat and mouse. Similar but less amounts of these granules were further revealed in the dorsal nucleus of the raphe of the rabbit, dog and cat. These granules positive for argentaffin or Schmorl's reaction were further tested with chromaffin, Gibbs's and azo-coupling reactions. Although the former two were negative, the last gave slightly positive reaction.

This substance was still capable of reducing ammoniacal silver or ferricyanide after the sections had been exposed to some of the blocking agents for SH groups and aldehydes. Shimizu *et al.*[12] recently showed histochemically the characteristic deposition of ascorbic acid in the locus cœruleus, which completely disappeared in the scorbutic guinea pigs. These granules were demonstrated with the argentaffin and Schmorl's reactions even in the scorbutic guinea pig's brain. These results seem to indicate that the reducing capacity of the granules is not ascribed to the presence of SH, aldehyde and ascorbic acid.

Furthermore, administration of a specific inhibitor of monoamine oxidase (1-phenyl-2-hydrazinopropane, catron, *JB*-516), 200 mgm./kgm., to normal rats, in which the granules could not be demonstrated, produced the appearance of abundant reducing granules in the locus cœruleus (Fig. 1*b*). This experiment indicates that this reducing substance might be the physiological substrate of monoamine oxidase in this locus.



(*a*) (*b*)

Fig. 1. *a*, Locus cœruleus of the guinea pig. Moderate amounts of argentaffin granules are seen within nerve cell bodies. Fixation with formalin. Fontana's argentaffin reaction. (× *c.* 450)
b. Locus cœruleus of the catron-administered rat. Showing the appearance of abundant granules. The animal was killed 4 hr. after subcutaneous administration of catron (200 mgm./kgm.). Fixation with formalin. Gomori's methenamine silver reaction. (× *c.* 450)

The present observations, together with the previous result of the pronounced monoamine oxidase activity in the locus[5], suggest that the reducing granules occurring in the locus cœruleus might consist of aromatic monoamine. It is likely that they could not be demonstrated by the chromaffin and Gibbs's reactions, because they occurred in low concentration and were scarcely recognizable.

The possibility that the locus cœruleus probably contains aromatic monoamine might also be supported by the recent physiological investigation by Megawa[13], which showed that the electrical stimulation of the locus has displayed the characteristic parasympathetic reactions. Brodie and Shore[14] suggested that serotonin acted as the chemical transmitter of the central parasympathetic system. This substance in the locus cœruleus seems to be easily demonstrable with fixation of formaldehyde and its reducing capacity seems to be weakened or abolished after fixation without formalin, a fact which resembles the enterochromaffin substance, serotonin. From our histochemical results and the physiological data of other investigators[13,14] it may be supposed that the locus cœruleus contains aromatic monoamine acting as parasympathetic transmitter.

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RADIOBIOLOGY

Reaction of Formaldehyde with Deoxyribonucleic Acid in Phage T1

In viruses the formaldehyde reaction takes place not only with the protein but also with the ribonucleic acid, as has been shown by Fraenkel-Conrat[1]. According to Staehelin[2] the binding of formaldehyde by ribonucleic acid appears to be a specific function of the amino-groups of the bases. Yet there is no formaldehyde reaction with amino-groups involved in hydrogen bondings, as is the case in the double-stranded deoxyribonucleic acid[2]. If this acid is subjected to dialysis with distilled water for several days in the cold (a procedure which is believed to break hydrogen bonds) it is rendered more reactive

towards formaldehyde[2]. This increased reactivity is reversible by addition of a buffer[2].

In concentrations lower than 1 per cent, formaldehyde is supposed to react with nucleic acid by forming monomethylol groups (—NH—CH_2OH), which can afterwards form methylene bridges by condensation[3]. The formaldehyde reactions in proteins have been discussed by Fraenkel-Conrat[3]. Heicken and Spicher[4] studied the inactivation and reactivation phenomena in phage *T*1 after formaldehyde treatment. Since amino-methylol groups are unstable[3], inactivated phages can be reactivated by storage in the presence of formaldehyde-binding media[4]. The reactivation-rate is low and does not disturb inactivation experiments if constant conditions for phage growth are used.

The following experiments were performed to decide whether the formaldehyde inactivation of *T*1 is caused by protein damage or by reaction with the deoxyribonucleic acid.

It is known that the ultra-violet survival curve of *T*1 is made up of two components of different ultra-violet sensitivities, where the lower sensitivity results from a reactivation of a certain fraction of inactivated phages, probably by the genome of the host bacterium[5,6]. This host cell reactivation requires an injection of the phage genome and can therefore be used to indicate that an inactivated phage still injects its deoxyribonucleic acid. It is further known that doses of ultra-violet light commonly used for inactivation experiments do not affect the adsorption and injection mechanisms of bacteriophages to an appreciable extent. If other inactivating agents permit a host cell reactivation at least involving the same number of inactivated phages as does ultra-violet light, it may be concluded that the inactivation results from damage in the phage genome and that the adsorption and injection mechanisms are not disturbed to any greater extent than after ultra-violet irradiation. If *T*1 is inactivated by a solution of formaldehyde in distilled water (*p*H 5·6), the survival follows an exponential function of the formaldehyde dose (Fig. 1, curve *A*). But if *T*1, inactivated under the same conditions, is pre-adsorbed on bacteria irradiated with ultra-violet light (*E. coli B*), the number of plaque-forming *T*1 is markedly decreased, since the reactivating mechanism within the bacteria is destroyed by the irradiation (Fig. 1, curve *B*); whereas the capacity of irradiated cells to yield progeny from untreated *T*1 is unchanged. This indicates that a great number of inactivated phages are reactivated by the host bacterium and that the formaldehyde reaction takes place in the genome. A comparison was made between the sensitivity of *T*1 towards formaldehyde and ultra-violet light, when in both cases the phage is propagated in bacteria irradiated with ultra-violet light, and the sensitivity towards formaldehyde and ultra-violet light when phages are grown in bacteria that perform a reactivation. After ultra-violet irradiation as well as after inactivation by formaldehyde, and within the experimental error, the ratio of total hits to those that cannot be reactivated is about 7 : 2 (Fig. 1, curves *B* and *C*). The similarity of the ratios makes it probable that the inactivation occurs in the same structure in the case of ultra-violet and formaldehyde treatment. As the reactivation is believed to be a result of recombination between homologous parts of phage and bacterial genome, equal ratios would be expected, if the protein damage after treatment with formaldehyde is negligible.

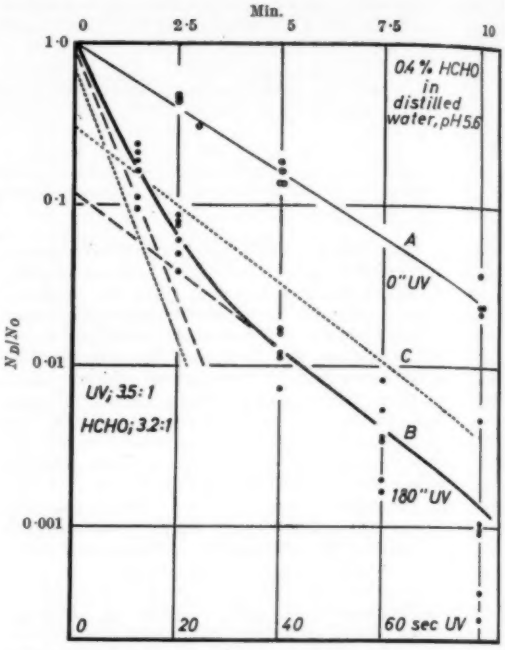

Fig. 1. Survival of formaldehyde-inactivated *T*1 in normal and ultra-violet irradiated *E. coli B*. Curve *A*, survival after pre-adsorption to unirradiated bacteria. Curve *B*, after pre-adsorption to bacteria irradiated by ultra-violet light, 180 sec. (a dose reducing the colony-forming ability of *E. coli B* by a factor of 10^4). Broken lines, decline and size of the two components of the survival curve *B*; ratio of declines 3·2 : 1. Curve *C*, dotted lines, decline and size of the two components of the *T*1-surviving curve after ultra-violet irradiation and propagation in untreated *E. coli B*; ratio of declines 3·5 : 1. Abscissa, time of incubation with formaldehyde and of ultra-violet irradiation respectively. Ordinate, relative number of surviving *T*1

But there exists still another possible explanation for the results in curve *A* and curve *B* of Fig. 1. In the case of inactivation by formaldehyde each phage which is injected survives by reactivation independently of the number of formaldehyde hits in its genome. Then, the inactivation measured in curve *A* must prevent the injection of the genome. By ultra-violet irradiation of the host bacteria, the ability for phage reactivation is lost in a certain number of cells and the damage by formaldehyde can be expressed (curve *B*). The ratio 3·2 : 1 would give the sensitivity of the total phage to the sensitivity of absorption and injection mechanisms. This explanation is very improbable, as it demands a survival of each injected phage independently of the number of formaldehyde hits in its genome.

According to the results of Fig. 1, formaldehyde hits and ultra-violet hits show the same relations concerning inactivation and host cell reactivation. Therefore, after inactivation by formaldehyde of *T*1 to 10 per cent or less survivors, the ultra-violet-surviving curve of the phages after subsequent irradiation should be an exponential function of the ultra-violet dose with the same slope as that of the 30 per cent fraction in the ultra-violet curve of untreated *T*1 (Fig. 2, curves *A*, *B* and *C*). (The steeper decline of the curves *B*, *C* and *D* in Fig. 2 is caused by the formaldehyde which has been present during irradiation in different concentrations[7].) Inactivation by formaldehyde to about 60 per cent survivors must yield an ultra-violet curve at subse-

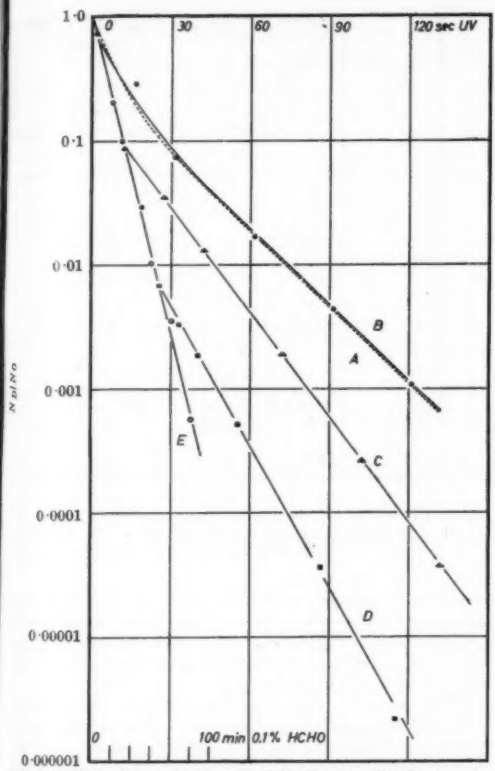

Fig. 2. Ultra-violet survival curves of *T*1 after preceding treatment with formaldehyde. Curve *A*, dotted line, ultra-violet survival curve of normal *T*1. Curve *B*, inactivation by formaldehyde to 65 per cent survivors and subsequent ultra-violet irradiation. Curves *C* and *D*, inactivation by formaldehyde to 9 per cent, respectively, 0·7 per cent survivors and subsequent ultra-violet irradiation. (The steeper decline of curves *C* and *D* results from an increased reaction-rate of formaldehyde with ultra-violet irradiated *T*1, ref. 7.) Curve *E* gives the survival of *T*1 after incubation with a 0·1 per cent formaldehyde solution in distilled water (*p*H 5·6) at 37° C. Abscissa, time of ultra-violet irradiation (for curves *B*, *C* and *D* the scale begins with the first point on the formaldehyde-inactivation curve); and time of treatment with formaldehyde respectively. Ordinate, relative number of surviving *T*1

quent irradiation, which is still composed of two fractions of different ultra-violet sensitivities (Fig. 2, curve *B*).

The results in Figs. 1 and 2 indicate that the inactivation of *T*1 by formaldehyde is mainly caused by a reaction with the phage genome. The fact that the ratio of reactivable to non-reactivable hits is the same in the case of formaldehyde and ultra-violet inactivation makes a formaldehyde reaction with the deoxyribonucleic acid most probable. The reaction has to occur with free amino-groups, which shows that not all possible hydrogen bonds are established in the deoxyribonucleic acid of *T*1.

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Effects of Ultra-Violet Light on Hydrogen Bonds in Deoxyribonucleic Acid of Phage *T*1

Phage *T*1, inactivated by formaldehyde, still injects its deoxyribonucleic acid, as can be shown by the degree of reactivation of 'formaldehyde-hits'[1]. The formaldehyde inactivation of *T*1 is believed to result from a reaction with the amino-groups of the bases in the deoxyribonucleic acid[1]. As Staehelin[2] has shown, formaldehyde in concentrations lower than 1 per cent reacts with free amino-groups of nucleic acids, but it does not react if these groups are involved in hydrogen bondings. Furthermore, the experiments of Nitschmann and Hadorn[3] make a formaldehyde reaction with the free imino-groups of the bases improbable.

Ultra-violet irradiation might cause a break of purine–pyrimidine hydrogen bonds in deoxyribonucleic acid. This break could be a non-lethal effect either in that it is reversible or in that it does not influence the viability of the phage. If ultra-violet light breaks hydrogen bonds in deoxyribonucleic acid, this would lead to an increased reaction-rate of formaldehyde with the amino-groups of the nucleic acid; but there should be no increase in phages with single stranded nucleic acid, if the reaction with the protein is not increased after ultra-violet irradiation. Presuming that a break of a hydrogen bond in the deoxyribonucleic acid is not necessarily lethal, the increased reaction-rate of formaldehyde with ultra-violet-irradiated phage deoxyribonucleic acid must lead to an increased inactivation-rate of irradiated

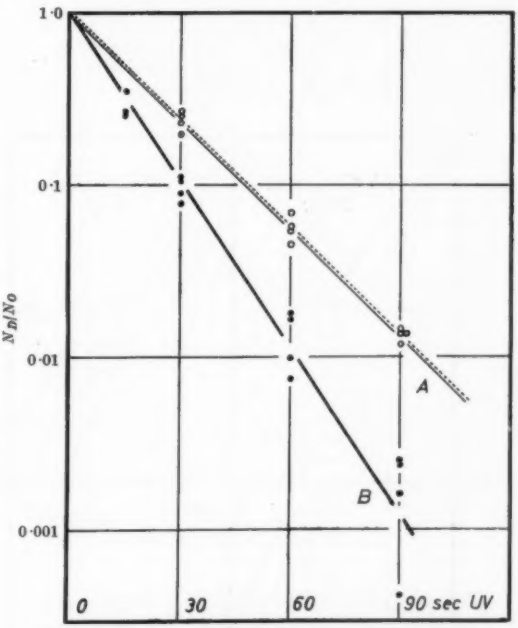

Fig. 1. Survival of *T*1 after irradiation with ultra-violet light. Dotted line, untreated *T*1, fraction which performs host–cell reactivation. Curve *A*, survival of *T*1 after pretreatment with formaldehyde and subsequent irradiation in buffer. Curve *B*, survival after irradiation in a 0·9 per cent formaldehyde solution in buffer. The steeper decline in the curve *B* does not result from an increased ultra-violet sensitivity, but shows the increased reaction-rate of *T*1 with formaldehyde after irradiation. Abscissa, time of irradiation; ordinate, relative number of surviving *T*1

$T1$ by formaldehyde ; and this increased inactivation-rate can easily be measured.

As a restitution of the hydrogen bonds might occur immediately after the break by ultra-violet light, the incubation with formaldehyde was performed during irradiation. In a first set of experiments, $T1$ was irradiated by ultra-violet light of wave-length 2537 Å. in the presence of 0·9 per cent formaldehyde in a phosphate buffer (*p*H 6·7, 0·02 *M* disodium hydrogen phosphate + 0·02 *M* potassium dihydrogen phosphate + 0·06 *M* sodium chloride). In these experiments the incubation times with formaldehyde were exactly the same for each sample of the different points in curve *B* (Fig. 1). In a second set of experiments, $T1$ was incubated at room temperature with 0·9 per cent formaldehyde in a phosphate buffer for the same time as the samples of the first experiments. Formaldehyde was then removed by a hundred-fold dilution in buffer, and the phages were afterwards irradiated (Fig. 1, curve *A*). These experiments were performed to show that formaldehyde given before irradiation does not alter the ultra-violet sensitivity of the surviving $T1$. The difference between curve *A* and curve *B* shows the increased inactivation-rate of irradiated $T1$ by formaldehyde. The number of amino-groups accessible for formaldehyde increases with greater doses of ultra-violet light, which indicates that hydrogen bonds are broken by the irradiation and that not each break leads to an inactivation of the phage.

The second aim was to determine whether the increased sensitivity to formaldehyde after ultra-violet irradiation is reversible, that is, whether the broken hydrogen bonds in the deoxyribonucleic acid rejoin. In these experiments, the phages were incubated for a given time with formaldehyde, at different times after ultra-violet irradiation. The curves drawn in Fig. 2 indicate that even after 24-hr.

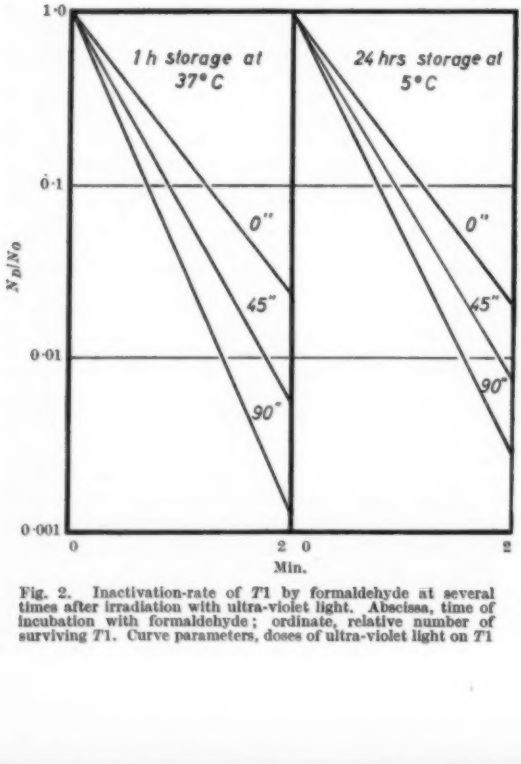

Fig. 2. Inactivation-rate of $T1$ by formaldehyde at several times after irradiation with ultra-violet light. Abscissa, time of incubation with formaldehyde; ordinate, relative number of surviving $T1$. Curve parameters, doses of ultra-violet light on $T1$

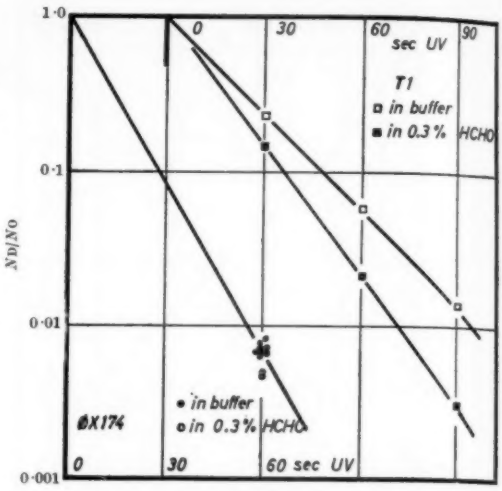

Fig. 3. Survival of φX 174 and $T1$ after irradiation in buffer and in a 0·3 per cent formaldehyde solution in buffer. Abscissa, time of irradiation; ordinate, relative number of surviving phages

storage in a phosphate buffer at 5° C. the increased formaldehyde-inactivation-rate of ultra-violet irradiated phages is still observed. (An increase of the reactivity of formaldehyde by the applied ultra-violet irradiation can be excluded.)

If the presumption should be correct that the rise in formaldehyde reaction-rate after ultra-violet irradiation is caused by a break of hydrogen bonds in the double-stranded deoxyribonucleic acid of $T1$, there should be no increase in the single-stranded deoxyribonucleic acid of φX 174. Therefore, $T1$ and φX 174 were irradiated in a buffer and, for comparison, in a 0·3 per cent formaldehyde solution. Fig. 3 shows an unchanged sensitivity in φX 174 but a significant increase in $T1$. This gives the additional result that there also is no increased inactivation from the formaldehyde reaction with the phage protein after ultra-violet irradiation, at least in φX 174.

As has been shown in Fig. 2, the hydrogen bonds broken by ultra-violet irradiation do not rejoin during a storage time of 24 hr. As it is improbable that a break of a single hydrogen bond alters the steric configuration of the deoxyribonucleic acid at this spot in such a way that the increased distance between the amino-group of one base and the ketone group of the complementary one does not allow a restitution of the hydrogen bond, a chemical change in the bases will be the cause. A possible mechanism might be the transition from the ketone- to a stable enole-configuration in the thymine, cytosine or guanine, since the enole-configuration prevents a rejoining of hydrogen bonds between the bases.

I wish to thank Miss U. Leukert for careful technical assistance.

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BACTERIOLOGY

'Oxidosomes' in Bacterial Anatomy

When bacterial cells are disrupted by any of the methods now in use, a considerable amount of ultramicroscopic particles is released from them, and these can be isolated by ultracentrifugation, usually for 1–2 hr. at 100,000*g*. This fraction is quite heterogeneous. The function and localization in the cell of its different components are not yet clearly known. This is probably the reason why the nomenclature of these particles is still in a rather confused state. According to different authors they have been called 'mitochondria', 'microsomes', 'particles', 'small particles', 'particulate fraction', '*ETP*', etc.

It seems to me that up to now two different groups of particles have been adequately characterized: the 'ribosomes' and fragments of the cytoplasmic membrane with high enzymatic activity which I propose calling 'oxidosomes'. It is hoped that a sharp delineation of both concepts will promote the eventual discovery of other types of particulate subunits of the bacterial cell.

Ribosomes[1] or ribonucleoprotein particles contain 40 or more per cent ribonucleic acid, and they appear to have little enzymic activity, except perhaps in protein synthesis. Their size ranges from 20 to 100 *S*. They are probably free-floating in the cytoplasm of the bacterial cell[2–5] and possibly identical to the granules seen with the electron microscope in ultra-thin sections[6]. A single cell of *Escherichia coli* is supposed to contain about 10^5 ribosomes.

The second group of particles, which has been studied to some extent, represents the small fragments from the solid cell envelope (probably the cytoplasmic membrane), and arises artificially during the disruption of the cells. They are enzymatically very active and contain, for example, many dehydrogenases and often the complete electron transport system (flavins, cytochromes, cytochrome oxidase) to oxygen. The latter particles are still designated by a variety of vague and often incorrect names, as mentioned above, and deserve an appropriate nomenclature. Since the main enzymatic function of the cytoplasmic membrane appears to consist in the oxidation of a large variety of substrates, I propose the name 'oxidosomes' for the particles derived from it. This name has been coined in analogy with other subcellular units, such as microsomes, lysosomes[7], sarcosomes, episomes[8], protosomes[9], chromosomes, ultramicrosomes, centrosomes and kinetosomes.

These oxidosomes have been studied in several bacteria. Mitchell and Moyle[10–12] showed that the cytochrome- and dehydrogenase-bearing oxidosomes from *Staphylococcus aureus* are derived from the protoplast membrane. The 'hulls' of *Azotobacter vinelandii* are fragmented into oxidosomes, containing cytochromes, hydrogenase and the oxidases for reduced triphosphopyridine nucleotide, reduced diphosphopyridine nucleotide, succinate and malate[3,4]. The particulate L-mandelic acid dehydrogenase of *Pseudomonas fluorescens*[13] is linked to oxidosomes, since Nakada and Nozu[14] recovered this enzyme in the 'ghost' fraction. The oxidosomes of *Acetobacter* have recently been studied extensively in this laboratory[15–19]. It was shown that 'protoplast' membranes contained the same enzymes as the particulate fraction[15] and that intermittent ultrasonic disruption fragmented the 'hull' of intact cells into the oxidosomes[16]. The latter contain the cytochrome system, several peroxidases[17], catalase[17], pyruvate decarboxylase, the oxidases for D- and L-lactate, acetaldehyde, ethanol and several other alcohols[18], glucose, gluconate, 2-ketogluconate, galactose, xylose, glycerol, several polyalcohols, reduced diphosphopyridine nucleotide, malate and succinate.

Except for the fact that oxidosomes can be isolated by ultracentrifugation at 100,000*g* for 1–2 hr. and look like small roughly spherical granules of a few hundred Å. in diameter[17] under the electron microscope, little is known of their physical properties. In view of their origin and formation, it seems possible that it is a group of particles with dimensions ranging from a few hundred Å. to the visible fragments of the 'protoplast' ghost.

Ultra-microscopic particles, containing cytochromes and other enzymes, have been isolated from several bacteria (for a review, see Alexander[20]). However, for most of them, their localization in the bacterial cell has not yet been established, and this problem needs re-investigation in view of the properties and the origin of the oxidosomes. It can be postulated, though, that most of them will be oxidosomes. It would furthermore not be surprising that it is a general rule that most, if not all, of the aerobic bacteria would contain nearly all their cytochromes and cytochrome-linked oxidase systems on the cytoplasmic membrane.

Work along this line with the strictly aerobic chemi-autotrophic micro-organisms is in progress in our laboratory.

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Interconnexion between the Protective Antigen and the Histamine-sensitizing Factor of *Bordetella pertussis*

The interconnexion between the protective antigen and the histamine-sensitizing factor of *B. pertussis* forms the subject of earlier reports[1–11]. Results agree in that the two factors, if not identical, are in any event closely related as regards both their site in the

bacterial cell[11] and their activity. We have studied the behaviour of the protective antigen and the histamine-sensitizing factor under conditions which may impair *pertussis* vaccines in the course of preparation. We produced 3-day-old cultures of each of six *B. pertussis* strains of phase I on solid semi-synthetic medium. After centrifuging them, the sediment was resuspended in buffered saline so as to obtain suspensions containing 500 × 10^9 cells/ml. The suspensions were divided in two parts: the first was treated with 0·3 per cent formalin and kept at 37° C. for 24 hr., the second with merthiolate (1 : 5,000) and heated to 56° C. for 30 min. Both kinds of suspensions were then stored at + 4° C. We prepared, moreover, suspensions from a mixture which contained all the six strains in equal proportions. These too were divided into formalin and merthiolate fractions (7 formalin, 7 merthiolate; 8 formalin, 8 merthiolate). Seven and 8 merthiolate seemed to be especially satisfactory in respect of protective potency and histamine-sensitizing activity: we prepared two precipitated vaccines from each of them. One contained 7 mgm./ml. of preformed aluminium phosphate gel (Holt[12]); a solution of trisodium phosphate was first added to the suspension in the case of the other vaccine and followed immediately by the admixture of a solution of aluminium chloride producing aluminium phosphate gel *in statu nascendi*. This vaccine contained likewise 7 mgm. of aluminium phosphate/ml. The addition of trisodium phosphate was observed to have raised the *p*H value of the mixture to 14. We determined the mouse-protection value of the suspensions and precipitated vaccines according to the prescriptions of the National Institutes of Health, Bethesda[13], and their histamine-sensitizing activity by the method of Preston[14]. It is evident from Table 1 that suspensions treated with merthiolate were significantly superior to those treated with formalin as regards both mouse-protective potency and histamine-sensitizing activity. The respective values of the two factors thus ran along parallel courses, and this close correlation was independent of whether old or fresh vaccines were employed. As regards precipitated vaccines, it is shown by our results that, however short their duration, strong alkalinity considerably impairs mouse-protective potency and histamine-sensitizing activity, while they are not affected by the preformed gel of Holt. Repeated tests confirmed these results in every respect.

Table 1. Mouse Protective Potency and Histamine-sensitizing Activity of Different *Pertussis* Vaccines

Strain and lot		Mouse protection test		Test of the histamine-sensitizing factor	
		ED_{50}	*RP**	SD_{50}	*RP*†
324*E*	(1F)	4,278	0·14	54	0·11
324*E*	(1M)	305·4	1·90	17	0·36
358*E*	(2F)	6,390	0·09	25	0·25
358*E*	(2M)	1,454·4	0·41	6·25	1·00
41405	(3F)	3,148·8	0·18	30	0·20
41405	(3M)	388·8	1·50	5·38	1·16
*CN*2894	(4F)	>3,000		21·6	0·28
*CN*2894	(4M)	926·4	0·64	5·4	1·15
*CN*2896	(5F)	2,319	0·25	45·5	0·14
*CN*2896	(5M)	401·4	1·50	2·5	2·50
*CN*2897	(6F)	2,319	0·25	21·6	0·28
*CN*2897	(6M)	179·4	3·30	4·2	1·45
546/4	(7F)	3,639	0·37	>50	<0·10
546/3	(7M)	1,054	1·30	6·25	1·00
*K*701*A*	(8F)	3,524·4	0·38	20	0·31
*K*701*B*	(8M)	519	2·60	2·2	2·88
546/3/1	St. n.	>3,000		>45	
546/3/2	Holt	228·3	2·60	11·25	
*K*64	St. n.	2,949	0·46	28	
*K*65	Holt	382	3·50	11·6	

Date of preparation of the vaccines. 1–6: VI (1959). 7: VI (1956). 8: X (1959). 546/3/1–2: V (1958). *K*64–65: XII (1959).
ED_{50} in million germs. Testing, I–II (1960).
SD_{50} in 1,000 million germs. Testing, II–III (1960).
* *RP*, relative potency referred to a lyophilized reference vaccine.
† *RP*, relative potency referred to the most frequently tested suspension 546/3, used also for the preparation of the lyophilized reference vaccine.
F, Formalin; M, merthiolate.

It was shown in earlier experiments[1,3] that *B. pertussis* of phase IV displays no histamine-sensitizing activity, and it is known to have no protective potency. We examined some factors which our experiments had shown to influence the protective antigen and histamine-sensitizing factor of *B. pertussis* in a similar manner. Similarity in their sensitivity to heat and in their response to different chemical agents and to variations in the phase of the bacterium has convinced us that the two factors, if not identical, are closely related and of a similar chemical structure; it is possible that they form a common antigen-complex. Since the mouse-protective potency and the histamine-sensitizing factor are affected in exactly the same manner by practically all factors which influence the efficacy of *pertussis* vaccines, it seems that—with the application of an adequate (perhaps lyophilized) standard vaccine—determination of the histamine-sensitizing factor might enable us to ascertain the real value of *pertussis* vaccines.

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BIOLOGY

Embryonic Mortality induced Experimentally in the Rabbit

Systemic treatment of the pregnant doe with suitable preparations, for example stilbœstrol, results in the destruction of the entire litter[1]. However, a surgical approach is required in order to destroy selected embryos. Huggett and Pritchard[2] induced prenatal mortality in rats by crushing the fœtuses with toothed forceps through the intact uterine wall at laparotomy. Later, Frazer[3] employed this technique to produce a standard number of implantations by destroying any in excess of the desired number. In rabbits, Brambell *et al.*[1] also used a surgical technique to kill embryos on the 16th or 19–20th days of pregnancy; in some animals attempts were made to kill the entire complement of embryos while in others only some of the embryos were treated. The technique

consisted of perforating the uterine swellings through the antimesometrial wall of the uterus and stabbing at the embryos with a fine suture needle. Rather surprisingly several of the embryos survived this treatment. During experiments on rabbits[1] concerning the distribution of embryonic death after implantation, it became necessary to ascertain the effect of the stage of pregnancy when death occurred on the resorption of dead embryos and their placentæ in litters that survived to term. Accordingly, a technique was developed whereby embryos could be killed selectively between the 7th and 17th days of pregnancy. It is the purpose of this communication to describe the application of this technique, which proved to be highly successful.

In 14 does laparotomy was performed under 'Nembutal' and ether anæsthesia, and the uterine horns were exposed. Then an incision about 4 mm. long was made through the antimesometrial wall of the selected uterine swellings, and the underlying membrane was also incised so that fluid would escape from the conceptus. In some cases very gentle digital pressure was applied to assist the escape of fluid, care being taken to avoid exteriorizing the embryo. In each animal, alternate uterine swellings were treated, but any sites that were considered to be undergoing resorption were not included. Altogether, 71 sites were treated in this manner either on the 7, 8, 10, 12, 15 or 17th day of pregnancy. Autopsy was performed on the 28th day of pregnancy, when the uterine contents were examined and weighed. Apart from one doe which was treated on the 15th day of pregnancy but aborted 7 days later, the remaining 13 does maintained the pregnancy. None of the sites which had been treated surgically contained a live fœtus at autopsy and with one exception death of the embryos appeared to have occurred shortly after the operation. When the operation was performed on or before day 15, the 62 treated sites were all represented by atrophic placentæ at autopsy. However, of the 9 sites treated at 17 days, only 2 were represented by atrophic placentæ, whereas the other 7 contained dead fœtuses. Six of these fœtuses were quite small and necrotic (crown rump length approximately 15 mm.) but the remaining one, which weighed 24 gm., probably survived the operation for at least 8 days. The weights of placentæ corresponding with treated sites are given in Table 1. Control placentæ associated with live fœtuses weighed 5–8 gm. on the 28th day of pregnancy.

It is thus apparent that the removal of fluid from the conceptus between days 7 and 17 prevents embryonic development in the rabbit. It would appear that during this period of pregnancy, when there is normally a peak of mortality[4], the embryo is more sensitive to environmental disturbances than at slightly later stages when it may continue to grow even after such severe operational procedures as decapitation[5]. No exact information is available on the interval elapsing between treatment and death of the embryo, but in one doe examined 2 days after the operation, which was performed on the 12th day of pregnancy, the treated embryos were no longer visible. This suggests that death and resorption take place very rapidly. However, judging from the amounts of placental tissues still present at the end of pregnancy it seems that the placenta may survive for some time after the embryo dies.

Table 1. Effect of Embryonic Death, induced Surgically between Seventh and Seventeenth Day after Mating, on the Weights of Placentæ recovered on the Twenty-Eighth Day of Pregnancy. (Means ± S.E.; Ranges in Parentheses)

Stage of pregnancy at operation (days)	Does No.	Uterine swellings		Weight (gm.) of placentæ corresponding to treated sites
		Total	Treated surgically	
7	2	23	11	0·7 ± 0·1 (0·3–0·9)
8	2	17	9	0·6 ± 0·1 (0·3–0·8)
10	3	28	15	1·1 ± 0·2* (0·3–1·7)
12	2	22	11	1·0 ± 0·1 (0·6–1·6)
15	3	37	16	2·6 ± 0·4† (1·5–4·4)
17	2	18	9	3·6 ± 0·2 (2·6–4·4)

* Based on 10 placentæ in 2 does; † based on 11 placentæ in 2 does.

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Photographic Studies of Fish Populations

Following the work of Veevers at Plymouth, we have been using cameras for some years for studies of sea-bed and mid-water life. These electronic flash cameras are suspended from a stationary or drifting research vessel, and operated either from the ship or by triggering at a set distance from the bottom. Our frogmen have also made studies of fishing gear and fish behaviour, though their work is limited to water of less than 10 fathoms depth and to conditions of full daylight.

We wished to extend our observations of fishing gear to deep water and to night, as well as daylight, conditions. We also felt it would be useful to take repeated photographs on one spot to study changing conditions.

We have therefore developed a simple self-contained camera and flash unit, using dry batteries and operated by a clockwork timer, which can take about 200 exposures at pre-set intervals—actually 1-min. and 3-min. intervals have been used up to the present.

This camera has been used in two ways:

(*a*) Attached near the headline of a trawl, so as to photograph the groundrope, or the zone immediately in front of it. The first sets of results, in depths up to 65 fathoms, confirm our frogmen's observations that fish in this region are normally swimming away from the groundrope and are therefore aware of the fishing gear. These observations will be continued to determine more exactly the influence of depth and light.

(*b*) Placed on a heavy tripod and left on the sea-bed for subsequent recovery by means of a buoy rope. When used in this way there is less chance of fish being disturbed from their normal behaviour than when the camera is swinging below a ship. A first set of experiments was made using this technique in March on Ballantrae Bank, in the Firth of Clyde. This showed that most of the fish then present near the bottom were herring, that their numbers and shoaling pattern on that occasion did not vary with the time of day

or night, and that the fish were present in small shoals of twelve to twenty fish. A local population density of about 2,000,000 fish per square mile was estimated from the known lens angle and limits of visibility.

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Influence of the Organic Matrix on Crystal Type in Molluscs

The calcium carbonate of mollusc shells occurs as calcite in some species, as aragonite in others, and certain species deposit calcite in one portion of the shell and aragonite in another portion. Various explanations have been suggested for the formation of aragonite, which is significantly less stable. Participation of the enzyme carbonic anhydrase and the influence of the protein matrix on which the crystals are deposited have both been suggested[1]. We have investigated the capacity of pieces of decalcified matrix to induce aragonite formation when inserted into a mollusc which normally forms only calcite, and also under *in vitro* conditions in which calcium carbonate is normally precipitated as calcite.

Protein matrix was prepared from shells of the fresh-water clam *Elliptio complanatus*, from the marine fan shell *Atrina rigida*, and from the nacreous layer of the Japanese pearl oyster *Pinctada martensii* by decalcifying with 1 per cent sodium ethylenediamine tetraacetate at *p*H 5·0. In each case the shell from which the matrix was taken was aragonite. Microscopic examination of each piece of matrix showed that all crystalline material had been removed. To make certain that submicroscopic crystals did not remain, some pieces of matrix were treated further with 0·01 *N* hydrochloric acid. For *in vivo* studies a single piece of decalcified matrix was wrapped around a fragment of glass cover-slip and inserted between the mantle and shell of the oyster *Crassostrea virginica*, which deposits a calcite shell. The temperature of the water was 19–25° C. The inserted pieces of matrix were removed after various intervals, usually 24 hr., washed thoroughly in deionized water, and the crystals identified under the polarizing microscope. *In vitro* calcification of matrix was carried out at 25 ± 2° C. in 100 ml. of a calcium bicarbonate solution prepared from reagent grade calcium carbonate in deionized water[2]. Pieces of decalcified matrix were placed in this solution for 25–44 hr., during which time the *p*H rose from 6·4 to 8·3.

Both *in vivo* and *in vitro*, aragonite crystals formed on protein matrix from aragonite shells (Table 1). Calcite crystals were also deposited on the matrix in both cases. The forms of calcite and aragonite crystals were distinct, the former being rhombic or rosette-shaped, and the latter irregular, spherulitic or hexagonal elongate. Aragonite was never found on substrates other than aragonite matrix. Tested substrates and conditions included: (1) glass cover-slip fragments inserted in *Crassostrea* (25 cases); (2) 'Formvar' plastic inserted in *Crassostrea* (4 cases); (3) decalcified calcite matrix from prismatic layer of *Pinctada* inserted in *Crassostrea* (3 cases); and (4) decalcified calcite matrix from prismatic layer of *Pinctada* tested *in vitro* (10 cases). Calcite crystals only were deposited in each of these instances.

Table 1

Test system	Source of protein matrix	Total No. of matrix pieces analysed	No. of matrix pieces with aragonite crystals
In vivo	*Pinctada martensii*	4	2
	Elliptio complanatus	8	1
	Atrina rigida	5	1* (?)
In vitro	*Pinctada martensii*	5	2†
	Elliptio complanatus	3	1‡
	Total	25	6 or 7

* The crystals were clustered and could not be analysed. Their form was irregular and different from calcite crystals.
† Final *p*H 8·3.
‡ Final *p*H 7·3.

Even though freshly secreted matrix might normally induce aragonite formation in aragonite-forming molluscs, aragonite induction might well have been prevented in the present experiments by at least three conditions: (1) alteration of the original state of the aragonite matrix; (2) conversion of small aragonite crystals to calcite after deposition; (3) presence of freshly secreted matrix by the calcite-forming host mollusc. The finding of aragonite in approximately one-quarter of the cases (Table 1) is accordingly of a higher frequency than might have been anticipated.

The results point to the protein matrix as a factor determining the crystal type in molluscs. At present we have no information on molecular spacing of protein matrix of calcite and aragonite shells which might permit profitable speculation as to possible mechanisms.

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The Skipper (*Scomberesox saurus*) in the Southern North Sea and the Thames Estuary

The skipper or saury pike is a pelagic fish of the open Atlantic and Mediterranean, ranging from Tristan da Cunha[1] northwards to the Gulf of Maine and the Bay of Fundy[2] in the western Atlantic, and to Christiana Fjord and Gjesvaer, near the North Cape, in the European Atlantic[3]. On the southern and south-western British coast it is of frequent, if not annual, occurrence, and catches of up to 100,000 have been reported at Mevagissey, Cornwall[4]. Farther north it has often been recorded from the Hebrides, the western Scottish coast and the Shetland and Orkney Islands[5], but the number of records decreases noticeably on the eastern coast of Scotland. Rae (personal communication) has found a specimen off Buchan Ness, Aberdeenshire (November 21, 1957); one in the Moray Firth (December 18, 1958); a shoal at Saltburn, Cromarty Firth (December 21, 1958); one at May Island, Firth of Forth (December 1, 1958); and one from a cod stomach 12′ N. St. Abbs, Berwick (February 18, 1959).

Farther south in the North Sea, records are still fewer. It has been noted from Scarborough[4] prior to 1884, and again in 1932[6], off Norfolk (October 1843)[4], and at Yarmouth[7], although it is probably more common than these few records suggest. In 1958 more than fifty were washed up at Southwold, Suffolk, and not less than twenty near Lowestoft (January 4–6, 1958); one at Corton (January 1); and one at Lowestoft (January 14) (R. W. Blacker, personal communication). In the Thames estuary, the skipper seems to have been recorded only once before, when one was found on the shore of West Mersea, Essex (December 20, 1907), as reported by Murie[8]. Writing of the occurrence of this fish on the Belgian coast, Poll[9] regarded it as rare, and in fact cited only two records, of a number on the coast in December 1854, and of one at Ostend in 1933. Kristensen[10] has, however, recorded more than a hundred in December 1955, stranded between Egmond and Texel (Holland), and three in January 1956 stranded at Den Helder, Egmond and Zandvoor. He also gives details of ten other records from the Dutch coast during the years 1909–52.

Records from various sources during the winter of 1959–60 indicate that the skipper entered the southern North Sea in larger numbers than previous records would suggest is usual. The following records have been made available to us by R. W. Blacker; two of a large number seen were caught (November 26) near Boston in the mouth of the River Witham; one alive at Corton (December 11); one alive on Lowestoft beach (December 28); two dead at Corton (December 29); a heavy stranding at Hemsby (December 31); one alive at Corton (January 1, 1960); two at Lowestoft, one at Gorleston (January 2); seventeen records between Walberswick and Yarmouth, mostly live strandings (January 2 to 12). Also during this period one of us (M. N. M.) obtained single live specimens from the River Crouch, Essex, on December 4 and 8, and a third one was found dead in the river on December 31. We have been informed by W. H. Harvey, fishery officer for the Kent and Essex Sea Fisheries Committee, that a number of skippers were caught in December by Southend and Leigh-on-Sea fishermen, while catching sprats near the East Cant Buoy, River Thames.

It seems likely from the records given here, and from those published by other authors, that in the late summer and early autumn there is often an invasion of the North Sea by shoals of *Scomberesox* through its northern entrance. Some, at least, of these fish turn southwards and may be caught later off the east coasts of Scotland and England. Others, reported in the Kattegat and the Oresund[11], probably represent a contingent entering the Baltic. Apparently only exceptionally do they move southwards as far as the Thames estuary. It is interesting that most of the skippers reported here were stranded, or were swimming in shallow water in the winter months of November, December and January, which seems to us to suggest that the winter cooling of the North Sea is associated with these strandings. A similar explanation has been advanced by Kristensen, who also considers that violent disturbance of the sea by storms may have some effect. In addition, he pointed out that exposure to low temperatures can result in disorientation in fish accustomed to warmer water.

We thank B. B. Rae, R. W. Blacker, W. H. Harvey and H. E. Jenner (through R. W. Blacker) for their co-operation in reporting catches of *Scomberesox*.

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Influence of Light Intensity on the Redox Potential in Leaves in cases of Iron-induced Chlorosis

AN investigation of the variation of the oxidation-reduction potential in cases of iron deficiency or calcareous chlorosis has been made in an attempt to find a relation between the redox general condition, the formation of the chlorophyll complex and its stability.

The phenomenon of iron-induced deficiency and its consequence, the low chlorophyll content, is thought to be the result of two effects[1]: (1) the low content of soluble iron; (2) the influence of light. The correction of the deficiency, that is, increasing the amount of chlorophyll present, can be obtained by injecting iron or by lowering light intensity[1]. After correcting the deficiency by one of the two processes, the green area of the leaf shows a redox potential higher than the normal characteristic of chlorotic material. However, the oxidation-reduction stage reached after the first process is permanent while that achieved by lowering light intensity is only temporary, since the potential returns again to that of the chlorotic conditions when a return to intense sunlight is made[2].

The existence of a higher redox stage after correcting the deficiency suggests the problem of discerning whether the increase of potential is a consequence or rather the cause of the formation of chlorophyll. The results obtained[2] have supported the second hypothesis, that is, that for raising the chlorophyll content a certain redox-level is needed below which the actual amount cannot be increased.

Study of variation in the redox potential when light intensity is changed is one of the points considered of main interest for the interpretation of iron-induced chlorosis.

The photolability of the chlorophyll complex formed with low light intensities has been ascribed[3] to the high amount of chlorophyll *b* formed, that is, to the different relation between the *a* and *b* forms of chlorophyll. But if the decomposition observed when reversing the conditions of light were only due to the different composition of the chlorophyll complex, it could be expected to stop such a decomposition as soon as the corresponding value for the green material had been reached. However, my results[2] do not con-

firm such assumption, since when the change from low to intense sunlight is made the decomposition continues, yet the relation between the two forms of chlorophyll shows a similar value to that of the green leaves. It means that the decomposition of the chlorophyll complex ought to be independent of the proportion of the *a* and *b* components.

Therefore it can be deduced that while the mechanism of chlorophyll formation is actually working in the chlorotic material, its protective mechanism on the contrary fails. The protection has been ascribed to reducing substances like polyphenols[3] since iron deficiency is reflected in the contents[1]. Now, if such interpretation were correct it is obvious that the formation of polyphenols ought to be prior to the formation of chlorophyll; but my results show that when iron is injected into the system following the diagnostic biological method[4] the formation of polyphenols occurs subsequent to the formation of chlorophyll.

According to this, the phenomenon of iron-induced deficiency and the protective effect of iron may be explained in the following way:

The chlorophyll present in chlorotic leaves is activated under the action of light, but only a small fraction will be stabilized while the rest decomposes. The reason is that in chlorotic conditions, ferric compound (protective agent) is present in small amounts, relative to the large number of electrons to be trapped. Then the iron will be mainly present in the ferrous form, and thence the redox potential will be low. If light intensity is lowered, only a small amount of chlorophyll will be activated and the small amount of ferric compound already present may not have at its disposal a sufficient number of electrons to pass to the ferrous form. In consequence, only very little ferrous compound will be present, and the redox potential will be higher than before. It can be easily realized that this stage cannot be permanent, because when restoring the high light intensity the conditions will be as in the beginning, namely, limited amount of ferric compound and high amount of activated chlorophyll will be present again. The only way to normalize the process is, therefore, by directly increasing the amount of ferric compound, for example, by injecting iron into the system.

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ENTOMOLOGY

A Fly Larva that tolerates Dehydration and Temperatures of −270° to +102° C.

The chironomid, *Polypedilum vanderplanki* Hint., breeds in small pools formed in shallow hollows on unshaded rocks in Northern Nigeria and Uganda. The hollows are often only 5–9 in. deep, and during the rainy season they may fill and dry several times. When the pools dry up the larvæ also dry up and remain in this condition until it rains again, when in about 1 hr. or so they resume feeding. The degree to which the larvæ dry up depends upon the relative humidity of the air. When moisture content was plotted against relative humidity, it was found that at 0 per cent relative humidity it was less than 3 per cent, at 60 per cent relative humidity it was 8 per cent, and at higher humidities the curve rose more steeply. In saturated air the moisture content altered from 3 to 33 per cent within 6 hr. at 25° C. The figures for moisture content are based on the assumption that the larva contains no water when its weight is constant at 106° C. The capacity to tolerate repeated dehydration is clearly of selective value to this species, and in the laboratory larvæ have survived as many as 10 dehydrations to a moisture content below 8 per cent. Between dehydrations they were allowed to feed in water, usually for 1–4 days.

Larvæ remain viable for long periods in the dry state. Larvæ kept at room temperatures and humidities for 39 months produced normal adults when allowed to grow in water[1]. For most of this period their moisture content was probably about 8 per cent; but in damp weather it may have risen to as much as 20 per cent. Recent attempts to activate 130 larvæ stored for 7 years and 127 larvæ stored for 10 years at room humidities were not successful. However, a high percentage of larvæ stored for 3 years at room humidities and then over calcium chloride for a further 7 years recovered temporarily when placed in water: hearts beat for several hours and the larvæ would respond to tactile stimuli.

Fig. 1. Skeletal muscles and tracheæ of the thorax of a living larva of *Polypedilum vanderplanki* that had been stored dry for ten years: three years at room humidities and a further seven over calcium chloride

It has been shown[2] that active larvæ tolerate water temperatures up to 41° C. for continuous periods longer than any they encounter under natural conditions. They do not survive exposure to temperatures of more than 43° C. for more than about 1 hr. The temporary pools in which the larvæ live have a bottom layer of mud in which the larvæ tunnel. When the pools dry, the larvæ remain in their tunnels and dry out with the mud. In the dry season the shade temperatures of some of the localities in which the species is found rise to a little above 40° C. The surface temperatures of fine soils may be 20–30° C. above shade temperatures. During the hottest part of the day temperatures at the surface of the dry mud may therefore be as high as 70° C., or perhaps a little higher owing to radiation from the rock sides of the hollows. However, the larvæ are probably rarely exposed to such temperatures because the surface of the mud is usually protected by some plant debris and most larvæ are 4–8 mm. below the surface. There is a steep fall in temperature away from the surface: even assuming a surface temperature of 80° C., the temperature at 4 mm. is 70° C. and at 8 mm. it is 60° C.[2]. When dried larvæ

were exposed to similar temperatures, it was found that 93 per cent survived exposure to 61° C. for 14 hr. and 26 per cent survived exposure to 68° C. for 11 hr.[2].

It has recently been found that some larvæ will grow and successfully metamorphose after exposure to 102–104° C. for 1 min. when dry. The moisture content of such larvæ was first reduced to less than 3 per cent with phosphorus pentoxide or other dehydrating agents. They were then placed in an electric oven at 53–55° C., and the temperature raised in 10 min. to 70° C. The larvæ were then dropped on to a watch-glass in another oven at 102–104° C. The temperature of the watch-glass in the second oven had reached equilibrium with the air temperature, so that no heat was lost from the larvæ by conduction. Larvæ exposed to temperatures of over 100° C. for as much as 5 min. did not live for more than 41 hr. when afterwards placed in water, but they will recover for a short time after exposure to 106° C. for 3 hr. or to 200° C. for 5 min.

Larvæ with a moisture content of about 8 per cent survive being dropped from room temperatures into liquid air (− 190° C.) or liquid helium (− 270° C.). Two lots of 5 larvæ each were dropped directly into liquid air and kept there for 2 and 77 hr. respectively. All the larvæ grew normally when afterwards placed in water. Larvæ were immersed in liquid helium in a glass tube inside a metal container. Five larvæ were exposed for 3 min. and six for 5 min. All eleven grew normally when placed in water. Some of the larvæ treated with liquid air and with liquid helium were kept and in due course metamorphosed.

Active larvæ die in less than 1 min. when immersed in absolute alcohol, but when their moisture content is reduced to about 3 per cent most recover. After 24 hr. in absolute ethanol, 27 of 33 larvæ recovered. Of these all but three died within 27 hr. One died after 24 days without feeding, one that was active but not feeding was fixed after 16 days, and one metamorphosed. Ten larvæ in absolute ethanol for 7 days all recovered, and the heart of one beat for more than 55 hr. Larvæ recovered for very short periods when kept in 95 per cent ethanol for 24 hr., but none recovered when the percentage of ethanol was as low as 90. Attempts to keep dry larvæ in pure glycerol were slightly more successful: three of four larvæ in glycerol for 24 hr. survived, as did two of six in glycerol for 67 hr. and one of twelve in glycerol for 7 days.

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Effect of amputating Stylets of Mature Apterous Viviparæ of *Myzus persicae*

While investigating the transmission of viruses by aphids, I observed that amputating the stylets of mature apterous viviparæ of *Myzus persicae* (Sulz.) caused them to give birth to an unusually large number of larvæ for aphids that could not feed. Indeed, for about a day the aphids produced as many larvæ as if they were feeding. To appreciate the unusualness of this response, it should be understood that, as a rule, keeping *M. persicae* without food almost completely inhibits the birth of larvæ. For example, when 25 mature adults were kept singly in small vials at about 20° C., the number alive after 1, 2 and 3 days was 25, 22 and 0 respectively; and during this time 14 of the aphids produced no larvæ, 9 produced one each, and 2 produced two each (13 larvæ in all). In comparison, the same number of aphids on a host plant produced more than 100 larvæ each day; and when 25 aphids were kept in vials after amputation of their stylets, each one produced from two to several larvæ (a total of 133 larvæ the first day); the bodies of these aphids became greatly distended, and all of them died on the second day, which was about a day earlier than the controls in vials died. The larvæ born in vials were nearly all alive at the end of the day when they were counted, and those transferred to host plants developed into adults within two weeks. Thus they appeared to be normal.

Afterwards it was found that certain other treatments of the mouth parts of *M. persicae* also caused mature aphids to deposit numerous larvæ when kept without food in vials. For example, cutting off about 25μ from the distal end of one of the mandibular stylets or from the interlocked maxillary stylets caused as marked a larviposition response as did removing all four stylets (Table 1). These operations also inhibited feeding, for aphids died within 2–3 days whether kept in vials or on a host plant. Those on plants sometimes made what looked like feeble attempts to probe, but they never had their stylets inserted when they were anæsthetized with the tip of the labium affixed to the plant. Thus they seemed to be unable to insert their stylets. The mandibular stylets were separated from the maxillæ by first sliding the labium towards the head to bare the stylets[1], then supporting the bared stylets on a microrod and gently pressing across them with a single hair. This operation by itself did not cause a larviposition response (Table 1); and since this method leaves the basal part of the stylets within the labial groove, the aphid can get its stylets back together simply by sliding the labium outwards as when it withdraws from feeding. But if the stylets are taken right out of the labial groove and then separated, *M. persicae* cannot get the mandibles back alongside the maxillæ and therefore cannot reinsert all the stylets into the labial groove. This operation therefore prevents further feeding, and interestingly enough it also causes the larviposition response. The same is true if the stylets are taken out of the labial groove and the labium mutilated. It is also interesting to note here that Johnson[3] obtained a larviposition response after decapitation of mature adults of *Aphis fabae* Scop. These various treatments that cause larviposition in the absence of feeding may not all do so in the same way, but it is noteworthy that they have at least one other thing in common in that they also permanently inhibit feeding. When an animal carrying fully developed young can no longer feed, there are obvious evolutionary advantages to a mechanism that allows the young to be born instead of perishing within the mother.

Table 1 also presents results to show that the larviposition response following amputation of the ends of the stylets was not simply a general response to wounding. Thus there was no such response after amputation of the antennæ, their tips, a tarsus, or a leg. Cutting off the antennæ so affected *M. persicae*

Table 1. Numbers of Larvæ produced by Mature Apterous Viviparæ of *M. persicae* kept Overnight in Glass Vials after Amputation of Certain Parts

Part amputated	Larvæ from ten aphids	
	Treated	Controls
All four stylets	44	0
End of one mandible (about 25μ)	47	0*
End of maxillæ (about 25μ)	38	0*
Both antennæ	1	0
Tips of antennæ	4	2
One tarsus	0	2
One leg	0	2

* Controls anæsthetized and mandibular stylets separated from maxillary stylets.

that those kept in vials died more than a day earlier than the controls in vials, and though the other operations did not affect the mortality of aphids kept in vials, presumably they caused as serious wounding as the removal of the ends of the stylets, which are believed to be non-living structures.

Cutting off the ends of the stylets has also caused a marked larviposition response by non-feeding aphids of all other species thus far tested. These were mature apterous viviparæ of: *Aphis fabae* Scop., *Macrosiphum granarium* (Kirby) (= *M. avenae* Fabr.), *Macrosiphum euphorbiae* (Thom.), *Myzus ascalonicus* Doncaster, and *Myzus certus* (Wlk.). This larviposition response suggests that the functions of the stylets are more complex than is generally thought, but any discussion of its significance at this time would be rather speculative. Further studies of this response are in progress and will be published elsewhere.

That cutting off the stylets of mature aphids causes them to produce young without feeding has been overlooked in earlier work. Thus Miller[3] claimed that cutting off the ends of the stylets did not prevent feeding by *Macrosiphum ambrosiae* (Thom.) or *M. granarium*. Apparently he concluded that the aphids fed because they reproduced and appeared to be feeding when viewed with a lens. In tests with *M. granarium* I found that amputating the ends of the stylets of mature apteræ caused a marked larviposition response by aphids kept in vials or on host plants. It is true that those on plants appeared to be feeding, but none could be found to have its stylets inserted; and furthermore, whether on plants or in vials the aphids died within two to three days. A second case in point is the following statement by Johnson[4], who was discussing the colonization of plants by alatæ. "It is interesting to observe that insertion of the stylets into plant tissues is not essential for either host selection or reproduction, and aphids with their stylets cut off will settle down and reproduce on suitable hosts just as aphids with intact stylets." Although this statement is quite true, it seems likely that after removal of the stylets the aphids would have settled down and reproduced on unsuitable plants and even on a non-plant surface.

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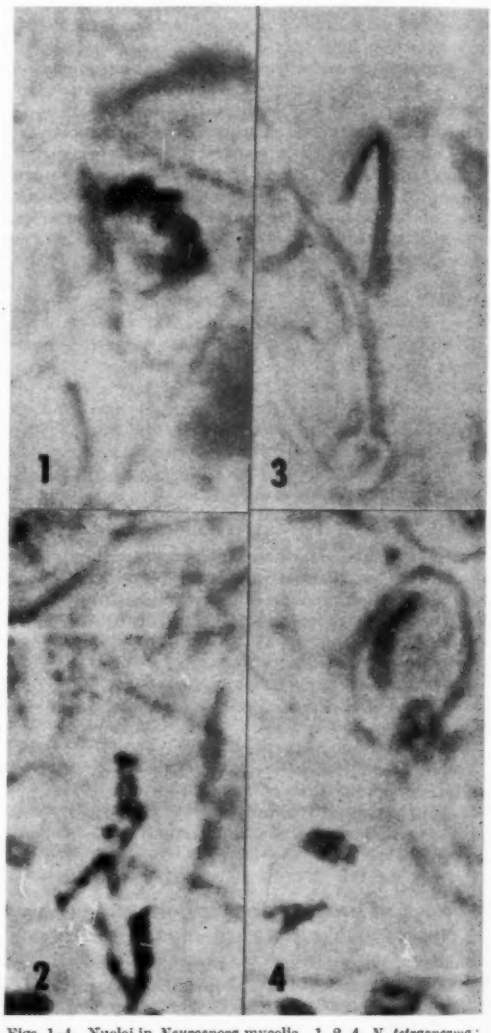
CYTOLOGY

Mitosis in *Neurospora*

In the ascus of *Neurospora*, meiosis and mitosis appear to resemble those in higher plants[1–4]. However, within the mycelium of *Neurospora* and of a number of other fungi, some observers report that the conventional stages of nuclear division are lacking, that the spindle is absent, and that the nuclei divide by becoming constricted and pulling in two[5–8]. We too have found that mitosis in *Neurospora* mycelium is unlike that in higher plants and animals and that there is no spindle; but we have observed also that the nuclei are filamentous (Figs. 1–4) and that they divide by splitting longitudinally.

The mycelium of three species of *Neurospora* Shear and Dodge was grown on a cover-slip, fixed,



Figs. 1–4. Nuclei in *Neurospora* mycelia. 1, 2, 4, *N. tetrasperma*; 3, *N. crassa*. 1, post-divisional nucleus with seven chromosomes; 2, above, post-divisional nuclei; below, dividing nucleus; 3, longitudinally dividing nucleus; 4, a loop and a spiral-shaped nucleus in the conidium before dividing, three V-shaped nuclei in the hypha. (× 1,750)

hydrolysed, stained in Giemsa and examined with a Zeiss microscope with 'Optovar' to give an initial magnification of 2,500. By this method, the chromosomes in the mycelium of *Neurospora tetrasperma*, *N. crassa* and *N. sitophila* were revealed. As in the ascus there are seven chromosomes in each species.

The chromosome groups most suitable for counting appear at first glance to be at metaphase, but in reality there are no such stages of mitosis in these nuclei. The chromosomes are arranged in linear series upon a filament (Fig. 1). McGinnis[9] has previously observed similar nuclei in the basidium of *Puccinia graminis*. In *Neurospora* the chromosomes are most easily counted when the filament is coiled within the nuclear membrane.

We have observed the following types of nuclei which we believe to be consecutive stages in mitosis: (1) a network within a spherical membrane; (2) an elongated network free of the membrane; (3) a narrow thread; (4) a thread longitudinally split (Fig. 3); (5) two separate daughter threads; (6) a shortened and thickened filament with distinct chromosomes; (7) a similar filament coiled within a membrane.

Masses of nuclear strands may occur in the lumen of hyphæ and also passing through their transverse septa. The identity of the individual nuclei is lost in the tangle (Fig. 2). In such masses, nuclear components may become interchanged, and hyphæ may become unlike genetically.

A detailed report of this work will be published elsewhere.

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Amphiplasty in *Ribes*

Several authors have reported that in *Ribes nigrum* L. (black currant) and *R. grossularia* L. (gooseberry), as in all other *Ribes* species so far investigated, a pair of satellited chromosomes is constantly present[1-3]. However, in 1957 it was noticed that only one satellite could be seen at metaphase in root tip cells of a black currant × gooseberry hybrid. Two further black currant × gooseberry hybrids have since become available for study and in both of these only one satellite is visible in root tip cells. The presence of two satellited chromosomes in two black currant and two gooseberry varieties (Baldwin and Boskoop Giant black currants, Broom Girl and Green Ocean gooseberries), parents of two of the hybrids mentioned above, has been confirmed. Evidently, in the hybrid, one of the parental satellites disappears. This phenomenon has been described in interspecific hybrids of *Crepis* by Navashin[4], who includes this and other chromosome changes due to hybridization under the term amphiplasty[4,5].

Further work in progress on amphiplasty in *Ribes* species hybrids will be reported elsewhere.

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GENETICS

A Screening Technique for Photosynthetic Mutants in Unicellular Algæ

In conjunction with experiments concerned with the genetics of photosynthesis in the sexually reproducing, unicellular, green alga, *Chlamydomonas reinhardi*[1] it became necessary to devise a technique to screen for mutants which were blocked in some phase of their photosynthesis. In the technique to be described here the inability to fix carbon dioxide in the light was taken as a requirement of a non-photosynthetic mutant.

There are forty-two mutants of *C. reinhardi* which cannot grow in the light unless the minimal medium[2] is supplemented with sodium acetate, and it was considered that certain of these mutants might not be able to fix carbon dioxide in the light. Rather than measuring carbon dioxide fixation individually for each mutant by the usual method[3] it was possible to assay for their ability to fix carbon dioxide while they were growing in colonies on agar medium contained in Petri dishes.

Petri dishes containing colonies of wild-type and several acetate mutants were inverted over a small watch-glass. Acetic acid (0·1 ml. of a 1 *N* solution) was pipetted into each watch-glass followed by the addition of 0·3 ml. of carbon-14 labelled sodium bicarbonate (2·5 μc./ml.). The operations were carried out in a hood. After the addition of the sodium bicarbonate the light from two 150-watt reflector floodlights was directed toward the Petri dishes. The light was allowed to remain on for 5 min. during which time a comparable set of colonies was exposed to carbon-14 labelled carbon dioxide in the dark as a control.

At the end of 5 min. the lights were turned off, and the colonies were replicated to filter paper. Each of the filter papers was exposed to the fumes of concentrated hydrochloric acid for 3 min. in order to drive off any unfixed carbon dioxide. Radioautographs were made of the replicated colonies using Kodak No-Screen X-ray film and were developed after exposure for one week. The ab lity of wild-type or a mutant to fix carbon dioxide was seen by the darkening of the emulsion of the film at a position which coincided with the position of the colony on the filter paper. The controls run in the dark gave negative radioautographs.

In this manner it was possible to screen rapidly the forty-two acetate-requiring mutants of *C. reinhardi* for their ability to fix carbon dioxide. Fig. 1*A* shows the filter paper replica of colonies of wild-type and seven different acetate mutants. Fig. 1*B* shows the radioautograph of the colonies. It appears from

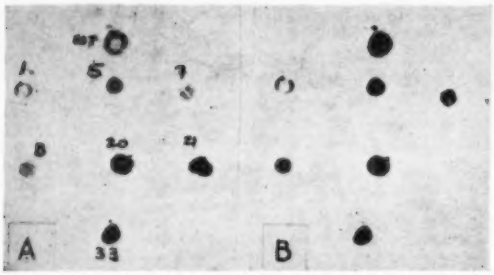

Fig. 1. Radioautographic technique used in screening for mutants which do not fix carbon dioxide. *A*, Filter paper replica of colonies which were exposed to $^{14}CO_2$ in the light; *B*, the radioautograph of the colonies. The wild type (*WT*) and seven acetate-requiring mutants are shown here. It is seen that *ac*-21 gives a negative radioautograph

the radioautograph that *ac*-21 does not fix carbon dioxide. Subsequent, more direct tests have revealed that the rate of carbon dioxide fixation in this mutant is some ten-fold less than that of wild-type and that the genetic block to photosynthesis lies in the process of photosynthetic phosphorylation[1].

Additional photosynthetic mutants have been obtained in the manner described here by screening colonies which had been derived from cells irradiated with ultra-violet light. Cells of wild-type were plated ($1{\cdot}5 \times 10^4$ cells per plate) to minimal agar which had been supplemented with sodium acetate. The cells were then irradiated with a dose which permitted 1 per cent survival. Following irradiation, the Petri dishes were placed in the dark for 12 hr., after which the colonies were allowed to develop at 25° C. and at a light intensity of 250-foot-candles from daylight fluorescent lamps. After 72 hr. the colonies were replica-plated to minimal agar supplemented with sodium acetate and to minimal agar supplemented with sodium acetate and yeast extract. When colonies had formed on these plates (usually within 72 hr. after replication) those growing on the medium supplemented with sodium acetate alone were tested for carbon dioxide fixation according to the method described above, while those growing on the medium supplemented with both sodium acetate and yeast extract were placed in the dark to await the results of the radioautographs. Those colonies giving a negative radioautograph were picked from the doubly supplemented medium in order to establish stock cultures. These in turn were tested for their inability to grow on minimal medium and to fix carbon dioxide as determined by more usual means.

The advantage of the technique described here lies in the rapidity and economy with which it is possible to screen large numbers of colonies for their ability to fix carbon dioxide. The technique should be feasible in any alga which can be handled in a manner similar to that of *C. reinhardi*. In addition, it should be possible to adapt the technique for most micro-organisms which can fix carbon dioxide when it is provided in the way described here.

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Elective Production of Thymine-less Mutants

Thymine-less (*thy*⁻) mutants of micro-organisms have not been easy to obtain. They die rapidly in the absence of thymine[1] so that, when penicillin is used to concentrate them, none survives the treatment. To discover them after mutagenic treatment requires direct detection methods and this, in turn, either good fortune[2,3] or a very high frequency of *thy*⁻ mutants among the survivors[4]. We have recently discovered a method whereby *thy*⁻ mutants of some strains of *Escherichia coli* 15 and K 12 can be produced in frequencies of the order of 50 per cent. Although no success was obtained with *Salmonella typhimurium*, the method seems to involve the general principle of differential growth-rates. With *E. coli* both the *thy*⁻ and *thy*⁺ strains are inhibited by aminopterin, but it seems that only in the *thy*⁻ can thymidine completely overcome that inhibition.

The strains used were *E. coli* 15 *h*⁻ (histidine-less), its colicine-resistant T-phage-sensitive derivative (15 *h*-CR [5]), and the wild type and various mutants of *E. coli* K 12 (a prototrophic, streptomycin sensitive derivative of HfrH, lysogenic for λ; W 1177 and CR 34, both of which are F⁻ and require threonine, leucine and vitamin B_1 although they differ in other genetic markers). The chemically defined medium employed was a modified Gray and Tatum medium[6]; the various strains were grown in this, supplemented as needed, with: L-histidine, 25 μgm./ml. (H^+ medium); DL-threonine, 200 μgm./ml. (Thr^+); L-leucine, 20 μgm./ml. (Leu^+); vitamin B_1, 0·2 μgm./ml. (B_1^+); thymine (Nut. Biochem. Corp.) 40 μgm./ml. (Thy^+); thymidine (Cal. Corp. Biochem. Res.) 20 μgm./ml. (Td^+).

For treatment the medium was also supplemented (*G*8 medium) with 12 of 14 substances the syntheses of which are thought to be inhibited by aminopterin[7] (thymidine and vitamin B_{12} were omitted, the latter for no good reason). The complete medium (*YECA*) consisted of a modified Gray and Tatum medium supplemented with 0·5 per cent Difco yeast extract, 0·5 per cent NZ-case and 0·5 per cent dextrose.

The treatment can be illustrated with the example of 15 *h*-CR. A single colony of this strain was inoculated into 5 ml. of *G*8 medium and incubated on a rotator at 37° C. overnight, during which time growth usually ceased at a level of 10^9 cells per ml. because of the exhaustion of dextrose (0·05 per cent). The cell suspension was diluted 10^{-1} and 0·1 ml. was added to 4·5 ml. of *G*8 medium along with 0·5 ml. of a solution of 2 mgm. aminopterin (Cal. Corp. Biochem. Res.)/ml. The mixture was incubated on a rotator at 37° C. for 24 hr., during which time the number of viable cells decreased to 10^2–10^3/ml. (*c.* 10^{-3} survival). Then 0·5 ml. of a solution of 2 mgm. thymidine (or thymine)/ml. was added and incubation continued, whereupon the cells began to grow. After 48–72 hr., full turbidity was achieved and the suspension was washed twice with saline, diluted and plated on *YECA* agar medium supplemented with 10 μgm. thymidine/ml. (*YECA*-Td^+). All *thy*⁻ mutants could not use thymine in the presence of *YECA* but would grow when thymidine was present. On chemically defined medium either compound supported growth equally well.

Colonies appearing on *YECA*-Td^+ agar medium were replicated on to H^+ and H^+Td^+ agar. Between 10 and 50 per cent were *thy*⁻ mutants, all retaining their *h*⁻ and (on the basis of limited tests) CR characteristics. When 0·5 ml. of a solution of 2 mgm.

5-bromo-deoxyuridine (Cal. Corp. Biochem. Res.)/ml. was also added to the G8 medium at the time of thymidine addition, up to 80 per cent of the population might be *thy*⁻.

Strain K 12 behaved somewhat differently from 15. Its derivatives grew more slowly in the chemically defined medium. When after some delay (1 day for HfrH and CR 34, 2 days for W 1177 and 3 days for wild type) they did reach full turbidity, a final concentration of 200 or 500 μgm. aminopterin/ml. was employed. Afterwards when thymidine and 5-bromo-deoxyuridine were added, it took 2 days for CR 34 and 4 days for W 1177, HfrH and wild type to reach full turbidity. The percentage of *thy*⁻ mutants present varied as follows:

	CR 34	HfrH	W 1177	Wild type
200 μgm. aminopterin/ml.	—	6·1	16	—
500 μgm. aminopterin/ml.	73	44	—	0·2

A preliminary analysis of a number of questions has been made with the following results: (1) The pre-treatment with aminopterin is unnecessary; (2) the appearance of *thy*⁻ mutants is not due to differential survival in aminopterin, because they appear without pre-treatment and die in the presence of aminopterin about as fast as *thy*⁺; (3) the *thy*⁻ mutants are the only ones produced by this treatment in detectable frequencies; the original markers of the treated bacteria are not observed to change in the *thy*⁻ produced—for example, *h*-CR bacteria remain *h*-CR; (4) the mutants produced are stable to an ordinary extent; recombination experiments with the K 12 strains indicate that the *thy*⁺ marker is transferred from the Hfr to the F⁻ parent, which result would seem to indicate that the genetic unit concerned is not extra-chromosomal; (5) the appearance of *thy*⁻ mutants seems not to be due to mutation induced by aminopterin but rather to the selective overgrowth after the thymidine has been added.

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STATISTICS

Time-Dependent Results for a Dam with Ordered Poisson Inputs

Let the content $0 \leqslant Z(t) < \infty$ of a dam at time $t \geqslant 0$, fed by an input $0 \leqslant X(t) < \infty$ in time t, and subject to a steady release at constant unit rate, be defined by the equation:

$$Z(t + \delta t) = Z(t) + \delta X(t) - (1 - \eta)\delta t \qquad (1)$$

where $\eta\delta t (0 \leqslant \eta \leqslant 1)$ is the time during t, $t + \delta t$ that the dam is empty. $X(t)$ is such that the arrival times of inputs form a Poisson process with parameter λ, the inputs themselves being of constant sizes α_1, α_2, and always arriving in this order (α_1, $\alpha_2 > 0$).

The process $Z(t)$ is Markovian when the number of inputs up to t is known. If we define:

$$F_i(z_0,z,t) = Pr\{Z(t) \leqslant z,\ X(t) = n(\alpha_1 + \alpha_2) + \delta_{i1}\alpha_1 | Z(0) = z_0\} \qquad (2)$$

for $n = 0, 1, 2, \ldots$; $i = 1, 2$; δ_{i1} being the Kronecker delta, the transition distribution function of the dam content is:

$$F(z_0,z,t) = Pr\{Z(t) \leqslant z | Z(0) = z_0\} = F_1(z_0,z,t) + F_2(z_0,z,t) \qquad (3)$$

It is readily shown the $F_i(z_0,z,t)$ satisfy differential-difference equations:

$$\frac{\partial F_i}{\partial t} - \frac{\partial F_i}{\partial z} = -\lambda\{F_i(z_0,z,t) - F_j(z_0,z-\alpha_i,t)\} \qquad (0 \leqslant z < \infty) \qquad (4)$$

where $i, j = 1, 2$ but $i \neq j$, and $F_i(z_0,z,t) = 0$ if $z < \max(0, z_0 - t)$; these are similar to Takács'[1] equation for the distribution function of the process in the case $\alpha_1 = \alpha_2$.

The Laplace–Stieltjes transforms of (4) lead to integral equations for the transforms of the $F_i(z_0,z,t)$, which also involve the probabilities of emptiness $F_i(z_0,0,t)$ $(i = 1,2)$. These do not appear to yield readily to solution. Direct probabilistic considerations will, however, lead to explicit results for the $F_i(z_0,z,t)$, and hence for the distribution function $F(z_0,z,t)$.

The probabilities of first emptiness $g_1(z_0,T_n)$ of the dam after n inputs starting with an input α_1, where $T_n = z_0 + [\frac{1}{2}(n+1)]\alpha_1 + [\frac{1}{2}n]\alpha_2 (n = 0, 1, 2, \ldots)$, are known. In some connected work, I have[2] obtained them recursively using a modified form of Kendall's[3] equation:

$$g_1(z_0,T_n) = \sum_{j=0}^{\infty} \sum_{i=0}^{1} e^{-\lambda z_0} \frac{(\lambda z_0)^{2j+i}}{(2j+i)!} g_{1+i}(j(\alpha_1 + \alpha_2) + \delta_{i1}\alpha_1, T_n - z_0) \qquad (5)$$

where $g_i(0,0) = 1$, $g_2(w,w + r\alpha_1 + s\alpha_2)$ is obtained from the formula for $g_1(w,w + s\alpha_1 + r\alpha_2)$ by interchanging α_1 and α_2 $(s - r = 0,1)$, and $g_i(w,T) = 0$ if $T < w (i = 1,2)$ for any $w > 0$, or $w = 0$ for any $T > 0$. A simpler evaluation of these by the method of truncated generating functions[4] is also given.

It is found that:

$$g_1(z_0,T_n) = \begin{cases} e^{-\lambda z_0} & (n = 0) \\ e^{-\lambda T_n} \dfrac{\lambda^n}{n!} z_0 \sum_{i=0}^{n-1} c_{ni} z^i{}_0 & (n \geqslant 1) \end{cases} \qquad (6)$$

where the coefficients c_{ni} can be obtained for any value of $n \geqslant 1$. In a recent paper Gani and Pyke[5] have also derived some simple inequalities for the $g_1(z_0,T_n)$ where $\alpha_1 > \alpha_2$.

Combinatorial arguments establish that:

$$F_i(z_0,0,t) = \sum_{r=0}^{\infty} g_1(t - r(\alpha_1 + \alpha_2) - \delta_{i1}\alpha_1, t) \qquad (7)$$

where $g_1(t - r(\alpha_1 + \alpha_2) - \delta_{i1}, t) = 0$ if $r(\alpha_1 + \alpha_2) > t - z_0 - \delta_{i1}\alpha_1$, and after a transformation ($u = z + t$, $t = t$), the equations (4) may be solved jointly step by step in the consecutive intervals:

$$[\tfrac{1}{2}(n+1)]\alpha_i + [\tfrac{1}{2}n]\alpha_j \leqslant z < [\tfrac{1}{2}(n+2)]\alpha_i + [\tfrac{1}{2}(n+1)]\alpha_j \quad (i, j = 1\ 2;\ i \neq j)$$

It is found that, in these intervals :

$$F_i(z_0,z,t) = \sum_{r=0}^{\infty} e^{-\lambda\{[\frac{1}{2}(r+1)]\alpha_i + [\frac{1}{2}r]\alpha_j - z\}} (\lambda\{[\tfrac{1}{2}(r+1)]\alpha_i + [\tfrac{1}{2}r]\alpha_j - z\})^r/r! \times \{\delta_{r,\left[\frac{r}{2}\right]} F_i(z_0,0,z+t-[\tfrac{1}{2}(r+1)]\alpha_i - [\tfrac{1}{2}r]\alpha_j) + (1-\delta_{r,\left[\frac{r}{2}\right]})F_j(z_0,0,z+t-[\tfrac{1}{2}(r+1)]\alpha_i - [\tfrac{1}{2}r]\alpha_j)\} \quad (8)$$

where $i, j = 1, 2$, but $i \neq j$. From these, the distribution function $F(z_0,z,t)$ of (3) can be obtained ; in the case $\alpha_1 = \alpha_2$, this naturally reduces to the form previously given by Gani and Prabhu[6].

The method outlined is equally applicable to the case of $p > 2$ ordered inputs $\alpha_1, \ldots, \alpha_p$. There is a corresponding increase in the complexity of the equations for the associated probabilities of first emptiness starting with an input α_1, as well as for the distribution function of the process.

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Plant Population and Crop Yield

Under the above title, Holliday[1] has recently published an equation which describes in a number of cases the relationship between yield and density in crop plants. In its simplest form this relationship is :

$$1/w = A\rho + B \quad (1)$$

where w is the yield per plant, ρ is the number of plants per unit area and A and B are constants. This equation was used (with these symbols) by Shinozaki and Kira[2] in 1956. An equivalent relationship connecting the total yield per unit area, $y = \rho w$, with the spacing per plant, $s = 1/\rho$, namely :

$$y = \frac{1}{A + Bs} \quad (2)$$

was proposed independently be De Wit and Ennik in 1958 [3]. These authors concede that the formula may not apply at high plant densities, and Holliday has directed attention to its limitations at very low plant densities, and accordingly has proposed a modification of equation (1). Appropriate modifications for both extremes are considered by Shinozaki and Kira[2]. Holliday further points out that even his modified equation cannot describe adequately total yield/plant density relationships where the form of the curve is parabolic, that is, where a certain plant population gives higher yields than either lower or higher populations. This type of curve, it is claimed, is typical of situations where yield is a function of reproductive growth, whereas curves that reach a stable maximum with increasing plant density are typical of yield of vegetative growth, and are defined by equations similar to or derived from equation (1).

There appear to be exceptions to this generalization ; thus the data of Wiggins[4] for the yield of threshed beans from soybean are adequately described by equation (1), whereas our own observations (unpublished) show that the yields of onions and lettuce frequently are not.

The Japanese contribution to knowledge of this subject does not appear to be widely known, and we therefore feel that the references given below[5] may be useful. Kira and his co-workers have shown how equation (1) can be derived from the logistic equation for plant growth together with certain other plausible assumptions, and have studied how the parameters in the equation change with time.

Hozumi, Kira and Shinozaki[6] have developed the application of equation (1) to cover the effects of any growth factor in suboptimal supply using the form :

$$\frac{1}{w} = \frac{A}{f} + B$$

where f is the amount of a factor supplied. They have developed the generalization for several factors varying simultaneously, using multilinear forms in the inverses of the amounts of the factors. Thus for two factors of amounts f_1 and f_2, they put :

$$\frac{1}{w} = \frac{A_1}{f_1} + \frac{A_2}{f_2} + \frac{A_{12}}{f_1 f_2} + B$$

and show that this model successfully describes the joint effect of light intensity and nutrient concentration on the growth of *Lemna minor*[7], and the joint effect of light intensity and density on the growth of *Hibiscus moscheutos*[6]. Although Kira and his co-workers have apparently successfully used equation (1) and their generalization of it for a number of species, we find that the relation :

$$\frac{1}{w^\theta} = A\rho^\theta + B \quad (3)$$

where θ is some positive quantity usually less than unity, frequently gives a strikingly better fit to data from spacing experiments. Equation (3) can be derived by analogous arguments to those of Kira *et al.*, the logistic equation being replaced by a generalization described by Richards[8]. By allowing the power of ρ in (3) to exceed that of w, a model can be obtained in which the yield per unit area shows a maximum at a certain finite density. A detailed account of this work will be published elsewhere.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 24

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. J. Valance-Owen: "Insulin Activity and Antagonism in Plasma".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. R. A. French: "Geography and Geographers in the Soviet Union".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. H. R. Rishworth: "Herbals and Herbalists, Ancient and Modern".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Cathodic Protection Panel Meeting (Brains Trust).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION, at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. A. Porter: "Southern Region, Kent Coast Electrification Scheme—Power Supply Apparatus" (Chairman's Address).

Tuesday, October 25

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. A. Armitage: "Some Observatories and Observers of the Seventeenth Century".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. H. Cooke-Yarborough and Mr. R. C. M. Barnes: "Rapid Methods for Ascertaining whether the Activity of a Weak Radioactive Sample Exceeds a Predetermined Level".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. C. H. Andrewes: "The Common Cold".* (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Stuart Hampshire: "Feeling and Expression" (Inaugural Lecture).*

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. F. S. Dainton, F.R.S.: "The Relationship Between Structure and Polymerisability of Monomers".

Wednesday, October 26

PHOTOBIOLOGY GROUP (at Queen Mary College, Mile End Road, London, E.1), at 11 a.m.—Report on work in progress in the Botany Department, Queen Mary College. Afternoon Session: Reports from the International Congress of Photobiology held in Copenhagen, July 31st–August 5th, 1960.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. J. H. Wilkinson: "Biochemistry of the Thyroid".*

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting. Wing-Commander E. W. Anderson, O.B.E.: "The Philosophy of Navigation Today" (Presidential Address).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. T. B. D. Terroni: "Channelling—a Sketch" (Chairman's Address).

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. Owen A. Saunders: "The Scientist's Contribution to Mechanical Engineering" (Presidential Address).

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion on "Analytical Aspects of Corrosion" opened by Mr. H. C. J. Saint.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Goldsmiths' College, Lewisham Way, London, S.E.14), at 7 p.m.—Mr. R. B. Collins: "Modern Colour Photographic Processes".*

Wednesday, October 26—Thursday, October 27

BRITISH INSTITUTION OF RADIO ENGINEERS (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 10.45 a.m. on Wednesday and 10 a.m. on Thursday—Symposium on "New Components".

Thursday, October 27

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. V. P. Whittaker: "The Subcellular Localisation of Neurohormonal Agents".*

UNIVERSITY OF LONDON INSTITUTE OF EDUCATION (in the Great Hall, Goldsmiths' College, New Cross, London, S.E.14), at 5 p.m.—Mr. W. G. Stone: "Some Possible Developments in the Youth Service".* (Final lecture in a series on "The Future of Education".)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. Hewish: "The Principles and Operation of Large Radio Telescopes".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Wilson Smith: "Virus Flocculation Reactions".* (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

CEMENT AND CONCRETE ASSOCIATION (at the Institution of Structural Engineers, 11 Upper Belgrave Street, London, S.W.1), at 6 p.m.—Mr. E. V. Finn and Mr. K. F. Shadbolt: "Structural Consideration of a New Animal Feed Mill in Belfast, Northern Ireland".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the BOROUGH POLYTECHNIC CHEMICAL SOCIETY, at the Borough Polytechnic, Borough Road, London, S.E.1), at 7 p.m.—Prof. C. W. Davies: "Some Aspects of Ion Exchange".

Friday, October 28

INSTITUTE OF BIOLOGY, PARASITOLOGY GROUP (in the Zoology Department, Birkbeck College, Malet Street, London, W.C.1), at 10.30 a.m.—Meeting on "Recent Advances in Fields of Parasitological Research".

LINNEAN SOCIETY and the BRITISH MYCOLOGICAL SOCIETY (in the Lecture Theatre of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 3 p.m.—Miss Gulielma Lister Centenary Meeting.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. Maurice Neville Hill: "Exploring the Ocean Floor".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (preferably with teaching and/or industrial experience) IN PHYSICS at the City College of Technology, Byrom Street, Liverpool 3, to teach to graduate level—The Director of Education, City of Liverpool Education Committee, 14 Sir Thomas Street, Liverpool 1 (October 28).

ASSISTANT IN ZOOLOGY—Secretary to the University Court, The University, Glasgow (October 31).

ASSISTANT LECTURER IN ZOOLOGY—The Registrar, The University, Leicester (November 1).

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Sheffield (November 5).

LECTURER IN PHARMACOLOGY—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (November 5).

LECTURER IN PSYCHIATRY—The Registrar, The University, Sheffield (November 5).

LECTURER (with research experience in physical oceanography or in an appropriate branch of physics or mathematics) IN PHYSICAL OCEANOGRAPHY at the Marine Biology Station—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (November 7).

RESEARCH ASSISTANT (with Ph.D. or equivalent research experience) FOR WORK ON THE ORGANIC CHEMISTRY OF FREE RADICALS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (November 7).

LECTURER (with a university degree in forestry and practical experience of forestry in Great Britain) IN THE DEPARTMENT OF FORESTRY—The Secretary, The University, Edinburgh (November 8).

ASSISTANT LECTURER (with medical qualifications registrable in this country) IN ANATOMY—The Registrar, The University, Manchester 13 (November 12).

DIRECTOR for an important RESEARCH STATION with a staff of over 30 engaged on ECOLOGICAL STUDIES—The Nature Conservancy (E), 19 Belgrave Square, London, S.W.1 (November 12).

VETERINARY PARASITOLOGIST (graduate in science or agricultural science or science with zoology as a major subject, or equivalent qualifications) AT THE McMASTER LABORATORY, Division of Animal Health, C.S.I.R.O., Sydney, Australia, to undertake general duties in parasitology, including studies of the ecology of nematode larvæ recovered from pastures and ruminal contents of sheep and the testing of new anthelmintics against nematode infection of sheep—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 202/179 (November 12).

BOTANIST (Plant Physiologist), Division A (with a good honours degree in botany or equivalent qualification, and at least two years research experience in the field of plant physiology) in the Botanical Division, Rubber Research Institute of Malaya, Kuala Lumpur, to investigate the occurrence and function of natural auxins and other growth factors in the rubber tree, *Hevea brasiliensis*—The Secretary, London Advisory Committee for Rubber Research (Ceylon and Malaya), Commonwealth Institute, Imperial Institute Road, London, S.W.7 (November 15).

CHAIR OF PHYSIOLOGY IN THE FACULTY OF MEDICINE, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 15).

SENIOR LECTURER or LECTURER IN VETERINARY PHYSIOLOGY AND BIOCHEMISTRY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 15).

LECTURER IN APPLIED MECHANICS; a LECTURER IN THERMODYNAMICS; and a LECTURER IN STRUCTURES—The Registrar, The University, Leicester (November 16).

LECTURER or ASSISTANT LECTURER (preferably with research interest in marine biology or comparative physiology) IN ZOOLOGY at

the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 19).

Official Fellow in Mathematics—The Principal, Hertford College, Oxford (November 26).

Research Fellow (Pharmacologist, Biochemist or Organic Chemist with biological experience, and of Ph.D. standard) for work in the field of Chemotherapy—The Secretary, Medical Research Council of Ireland, 50 Merrion Square, Dublin (November 26).

Assistant Lecturer in Physical Chemistry—The Registrar, Massey Agricultural College (University of New Zealand), Palmerston North, New Zealand (November 30).

Chair of Pure Mathematics—The Registrar, University College, Singleton Park, Swansea (November 30).

Lecturer or Assistant Lecturer in Geography at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

Professor of Agriculture and Director of the College Farm—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (December 1).

Associate Professor of Medicine at the University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia and London, December 12).

Chair of Philosophy at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, December 31).

Assistant Experimental Officer (familiar with elementary chemical techniques and possessing H.N.C. or equivalent, or, if under 22, qualifications leading to the above) in the Chemical Microbiology Department for work on cheese flavour—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 60/15.

Assistant Lecturer (graduate in chemistry, and preferably with experience in the food industry) for duties which will include the Teaching of Chemistry and Food Quality Control—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

Biochemical Technician (preferably with experience of microbiological techniques)—Head of the Department of Physiology and Pharmacology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

Biochemist (whole-time, non-resident) for hospital biochemical work in the Mansfield Area Pathology Service—The Hospital Secretary, King's Mill Hospital, Sutton-in-Ashfield, Notts.

Broadcasting and Film Liaison Officer (with a good general scientific background, familiarity with 16 mm. film production methods and equipment, and some knowledge of broadcasting techniques) at the Headquarters of the Department of Scientific and Industrial Research, London, S.W.1, for duties which will be concerned mainly with liaison between research organizations and film companies. The work also includes projection and care of equipment, selection and editing of film for T.V. use and promotion of films—Manager (P.E. 3158), Ministry of Labour, Professional and Executive Register, Atlantic House, Farringdon Street, London, E.C.4.

Chemist or Biochemist (with a good honours degree in science or equivalent qualification) to undertake research on the quality of protein in animal feeding stuffs—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen.

Geneticist, Principal Scientific Officer or Senior Scientific Officer grade (with Ph.D. or a good honours degree with at least two years postgraduate research experience in microbiology) to take over the Genetics Section—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

Laboratory Technicians and Instrument Makers for established posts in Research and Teaching Laboratories concerned with electronics, vacuum physics, ultrasonics, X-rays, solid state physics, etc.—Head of the Physics Department, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Microbiologist (with research experience) for work on Radiation-induced Mutations in Microbial Populations—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell.

Organic Chemist (with an honours degree in chemistry or biochemistry) to assist with research into the chemistry of micro-organisms—Prof. J. H. Birkinshaw, Department of Biochemistry, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1.

Physical Metallurgist or Physicist (graduate, with research experience) in the Physical Metallurgy Division, Department of Mines and Technical Surveys, Canada, to work with a group carrying out fundamental investigations in metal physics—Dept. D.M.T.S., National Employment Service of Canada, 61 Green Street, London, W.1.

Reader in Analytical Chemistry—The Academic Registrar, Loughborough College of Technology, Loughborough, quoting 14/2/AF.

Research Fellow (with a higher degree or research experience), or an Assistant (with a degree in chemistry or physics) in Polymer Chemistry for work of a physicochemical nature—The Registrar, Bradford Institute of Technology, Bradford, 7.

Research Fellows and Research Assistants (with a good honours degree in pharmacy or other allied subjects) in the Department of Pharmacy—The Registrar, Bradford Institute of Technology, Bradford 7.

Research Fellows (with a first- or second-class honours degree, evidence of high standard of ability in research, and at least two years postgraduate research experience, three years for senior fellows) at Government Scientific Establishments, in a very wide range of topics, especially in the physical sciences and in various branches of biology—The Civil Service Commission, 17 North Audley Street, London, W.1, quoting S/5060/60.

Technician (preferably with experience in microbiology) in the Science Department, to be responsible for the preparation of class materials and maintenance of stock cultures, and general duties—The Principal, Ewell County Technical College, Reigate Road, Ewell, Epsom, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Rubber Producers' Research Association. Twenty-second Annual Report. Pp. 53. (London: British Rubber Producers' Research Association, 1960.) [28

Bwrdd Gwarchodaeth Natur Cymru. The Nature Conservancy. Pp. 12. (Bangor: The Nature Conservancy, Penrhos Road, 1960.) [28

Middlesex County Council Education Committee. Evening Classes: a Guide to Facilities in Middlesex, 1960–61. Pp. 50+4 plates. (London: Middlesex County Council Education Committee, 1960.) [28

Department of Scientific and Industrial Research. Forest Products Research 1959: Report of the Forest Products Research Steering Committee, with the Report of the Director of Forest Products Research. Pp. vi+62+12 plates. (London: H.M. Stationery Office, 1960.) 5*s.* 6*d.* net. [28

Electing the American President. By Keith Kyle. Pp. 25+iii. (London: The Commonwealth-American, Current Affairs Unit of the English Speaking Union, 1960.) [28

Ministry of Agriculture, Fisheries and Food. Farm Buildings Pocketbook: Some Useful Advice giving Standards, Dimensions and Data for those interested in the Design of Farm Buildings. Pp. iv+43. (London: H.M. Stationery Office, 1960.) 2*s.* net. [28

Other Countries

Instituto Antartico Argentino, Buenos Aires. Contribucions. No. 28: La Campana Antartica Invernal del Rompehielos "General San Martin" en 1957. Por Jorge A. Boffi. Pp. 15. No. 40: Observaciones Ionosfericas Argentinas en el Antartico. Por Jose A. Rodriguez. Pp. 11. No. 42: La Campana Antartica 1941–42. Por Alberto J. Oddera. Pp. 15. No. 43: La Campana Antartica 1942–43. Por Silvano Harriague. Pp. 12. No. 44: La Campana Antartica 1958–59. Por Adolfo A. R. Schulze. Pp. 13. (Buenos Aires: Instituto Antartico Argentino, 1959.) [267

United States Department of Health, Education and Welfare: Public Health Service. Publication No. 761: Activities of the National Institute of Health in the Field of Gerontology—Research Grant and Training Projects Active on January 31, 1960, and Intramural Research Projects Carried on during calendar year 1959. Pp. v+41. (Washington, D.C.: Government Printing Office, 1960.) 30 cents. [267

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 35: Plane Coordinate Intersection Tables ($2\frac{1}{2}$ minute), Oregon. Pp. iii+166. (Washington, D.C.: Government Printing Office, 1960.) 1 dollar. [267

Department of Fisheries, Ceylon. Fisheries Research Station Bulletin No. 9: Lobster Fishing in Ceylon. By G. H. P. De Bruin. Pp. 18. (Colombo: Fisheries Research Station, 1960.) [267

Engineering Experiment Station of the University of Illinois, Urbana. Engineering Departmental Reports and Theses 1958–1959: Abstracts of Engineering Departmental Reports and Titles of Theses of the University of Illinois College of Engineering. Edited by James C. Ballowe and Paul Bryant. Pp. 47. Bulletin No. 456: Titania Opacified Porcelain Enamels. By R. D. Shannon and A. L. Friedberg. Pp. 49. Circular No. 68: A Comparison of the Performance of Various Room Heating Units used in the I-B-R Research Home. By Warren S. Harris and Loren N. Montgomery. Pp. 27. A Summary of Engineering Research, 1959. Compiled and Edited by W. David Penniman, James C. Ballowe, R. Alan Kingery and Paul T. Bryant. Pp. 146. (Urbana, Ill.: University of Illinois, 1960.) [267

Hokkaido University, Sapporo, Japan. Monograph Series of the Research Institute of Applied Electricity, No. 8: Some Developments in Network Theory. By Prof. Y. Asami. Pp. iii+146. (Sapporo: Research Institute of Applied Electricity, 1960.) [28

Tokyo Astronomical Observatory. Tokyo Astronomical Bulletin. Second Series. No. 121 (November 20, 1959): Precise Measurement of Travel Times of Radio Signals between Europe and Tokyo. (Potsdam versus Tokyo) 1. By S. Iijima, K. Futsukaichi and Goro Shibutani. Pp. 1409–1495. No. 124 (February 10, 1960): Discussion of Occultations Observed in Japan with the Photoelectric Equipments from 1954 to 1958. By S. Aoki and Y. Endo. Pp. 1515–1524. No. 126 (March 5, 1960): Polar Tube Observations during 1959. By N. Sekiguchi and J. Matsumoto. Pp. 1537–1538. No. 127 (March 15, 1960): Approximate Positions of Artificial Satellites Obtained with the Baker-Nunn Schmidt Camera (2). Pp. 1539–1553. No. 130 (June 15, 1960): Approximate Positions of Artificial Satellites Obtained with the Baker-Nunn Schmidt Camera (3). Pp. 1621–1643. (Tokyo: Tokyo Astronomical Observatory, 1959 and 1960.) [28

United States Naval Observatory. Circular No. 89: Solar Eclipses, 1968–1970. By Simone Daro Gossner. Pp. 8. (Washington, D.C.: United States Naval Observatory, 1960.) [28

National Academy of Sciences: National Research Council. NAS-NS 3010: The Radiochemistry of Barium, Calcium, and Strontium. By D. N. Sunderman and C. W. Townley. Pp. vi+118. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960.) 1.25 dollars. [28

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